Cancer Treatment Reports

A Publication of the National Cancer Institute

Volume 71
Number 3
March 1987

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES Public Health Service National Institutes of Health

Cancer Treatment Reports

National Cancer Institute, Vincent T. DeVita, Jr, ***Director***

International Cancer Information Center
Susan Molloy Hubbard, ***Director***

Publications Branch
Robin A. Atkiss, ***Chief***

Robert E. Wittes, ***Editor-in-Chief***
Dee Kittner LeRoy, ***Managing Editor***

Editorial Board

Joseph Aisner
Lewis Aronow
Gregory Curt
Richard L. Cysyk
Ross C. Donehower
Michael Friedman
Timothy J. Kinsella
Robert F. Ozols
Carl Pinsky
David G. Poplack
Richard Simon

Advisory Board

Charles M. Balch
Robert C. Bast, Jr
Gianni Bonadonna
Edwin C. Cadman
Giulio J. D'Angio
I. David Goldman
Heine H. Hansen
Simon Kramer
Susan E. Krown
John E. Niederhuber
T. L. Phillips
H. M. Pinedo
Carol S. Portlock
Joseph V. Simone
Samuel A. Wells, Jr

Editorial Staff

Editors:
Elaine Price Beck
Mary Olvey Cowgill

Editorial Assistant:
Julia Redmond

Manuscript Office:
Olga Sisson

Editorial Policy

The Editorial Board of *Cancer Treatment Reports* will consider manuscripts on original observations in laboratory and clinical research from all scientists. A manuscript is considered for publication with the understanding that it has not been published previously and is submitted exclusively to *Cancer Treatment Reports.* Opinions expressed by the authors are not necessarily those of the publisher or its editors. Scientific contributions in any of the following areas of investigation are solicited: surgery, radiotherapy, chemotherapy, immunotherapy, supportive care, pharmacology, cell biology and kinetics, mechanisms of drug action, and medicinal chemistry.

Submission of Manuscripts

Manuscripts and all inquiries concerning the status of manuscripts should be addressed to: Managing Editor, Cancer Treatment Reports, R. A. Bloch International Cancer Information Center, Bldg 82, Rm 231, National Cancer Institute, Bethesda, MD 20892 (301-496-6975). All material submitted for consideration in the journal will be subject to review when appropriate by at least two outside reviewers and one member of the Editorial Board. The decision to publish a manuscript is solely the responsibility of the Editorial Board. Prospective authors should consult the "Instructions to Authors."

Copyright

Publication of manuscripts is contingent on assignment of copyright to the National Cancer Institute. Forms for this purpose will be sent to authors when a manuscript has been approved for publication. All material published in this journal is in the public domain; therefore, permission to reproduce any portion is not necessary. As a matter of courtesy, contact should be made with the author concerned.

Subscriptions

Cancer Treatment Reports is for sale by the Superintendent of Documents, US Government Printing Office (GPO), Washington, DC 20402; a subscription form appears at the back of most issues. The subscriber service desk of GPO may be reached directly at (202) 275-3054 for any questions concerning individual subscriptions.

The Secretary of Health and Human Services has determined that the publication of this periodical is necessary in the transaction of the public business by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget.

INSTRUCTIONS TO AUTHORS

Title Page
Includes complete title of the manuscript and the full name, affiliation, and address of each author. Indicate full name and address of author to whom correspondence and/or reprint requests are to be sent.

Summary
Begin the article with a short summary.

Text
Submit an original and 3 copies of the manuscript, typed double-spaced. Number each page consecutively. Dot-matrix or italicized texts will not be accepted. Use accepted spelling, abbreviation, and hyphenation found in *Webster's New International Dictionary.* Footnotes should be typed at the bottom of the page on which they appear.

Tables
Submit each table on a separate page, typed double-spaced. Please use only standard abbreviations; otherwise use full words.

References
Submit references typed double-spaced. Follow the standard form of *Index Medicus:* author, title, name of journal, volume number, page or inclusive pages, and year. In book references give author, chapter title, book title, city and name of publisher, date of publication, volume number, and pages cited. References in text should begin with number 1 and continue consecutively. References to papers published in meeting proceedings should include author; title of paper; title of meeting; city and name of publisher; date of publication; volume number; and pages. Papers presented at meetings but not published should be cited in a footnote rather than in the reference section.

Illustrations
Photographs and graphs should be clear, glossy prints. If original artwork is submitted, it should be done in black ink for good reproduction. Please send 2 copies of each print. Photocopies reproduce poorly. Color photographs or transparencies are acceptable.

Nomenclature
Names of compounds should conform to American usage. See *USAN and the USP Dictionary of Drug Names* or *The Merck Index* for proper nomenclature.

Brief Reports
Manuscripts published in the Brief Reports section should be no longer than 5 double-spaced pages including references, tables, and a short summary; illustrations are usually omitted.

Clinical Trials Summaries
Manuscripts published in this section should be no longer than 300 words and should be submitted with no more than 1 table and 5 references, and no illustrations. Tables should include: *patient characteristics and prognostic factors* (age/sex, performance status, stage of disease, pathologic types of disease, prior therapy), *drug regimen,* or *standardized toxicity grades* to demonstrate that an appropriate, biologically active dose was administered.

Letters to the Editor
Letters to the Editor should be no longer than 3 double-spaced text pages and should be submitted with no more than 1 table and 5 references; figures are usually omitted.

Reprints
Supplied free of charge, 100 reprints (approximately 20 reprints for Letters to the Editor) will be sent to the senior or specified author about 3 weeks after publication of the article.

Announcements
Announcements of meetings are accepted for publication free of charge. Mail to: Editorial Office, Cancer Treatment Reports, R. A. Bloch International Cancer Information Center, Bldg 82, Rm 231, National Institutes of Health, Bethesda, MD 20892. Allow 4 months for publication.

METHODOLOGIC GUIDELINES FOR REPORTS OF CLINICAL TRIALS*

1. Authors should discuss briefly the quality control methods used to ensure that the data are complete and accurate. A reliable procedure should be cited for ensuring that all patients entered on study are actually reported upon. If no such procedures are in place, their absence should be noted. Any procedures employed to ensure that assessment of major endpoints is reliable should be mentioned (eg, second-party review of responses) or their absence noted.

2. All patients registered on study should be accounted for. The report should specify for each treatment the number of patients who were not eligible, who died or withdrew before treatment began. The distribution of follow-up times should be described for each treatment, and the number of patients lost to follow-up should be given.

3. The study should not have an inevaluability rate for major endpoints of greater than 15%. Not more than 15% of eligible patients should be lost to follow-up or considered inevaluable for response due to early death, protocol violation, missing information, etc.

4. In randomized studies, the report should include a comparison of survival and/or other major endpoints for all eligible patients as randomized, that is, with no exclusions other than those not meeting eligibility criteria.

5. The sample size should be sufficient to either establish or conclusively rule out the existence of effects of clinically meaningful magnitude. For "negative" results in therapeutic comparisons, the adequacy of sample size should be demonstrated by either presenting confidence limits for true treatment differences or calculating statistical power for detecting differences. For uncontrolled phase II studies, a procedure should be in place to prevent the accrual of an inappropriately large number of patients, when the study has shown the agent to be inactive.

6. Authors should state whether there was an initial sample size and, if so, what it was. They should specify how frequently interim analyses were performed and how the decisions to stop accrual and report results were arrived at.

7. All claims of therapeutic efficacy should be based upon explicit comparison with a specific control group, except in special circumstances where each patient is his own control. If nonrandomized controls are used, the characteristics of the patients should be presented in detail and compared to those of the experimental group. Potential sources of bias should be adequately discussed. Comparison of survival between responders and nonresponders does not establish efficacy and should not generally be included. Reports of phase II trials which draw conclusions about antitumor activity but not therapeutic efficacy generally do not require a control group.

8. The patients studied should be adequately described. Applicability of conclusions to other patients should be carefully dealt with. Claims of subset-specific treatment differences must be carefully documented statistically as more than the random results of multiple-subset analyses.

9. The methods of statistical analysis should be described in detail sufficient that a knowledgeable reader could reproduce the analysis if the data were available.

**From* Methodologic Guidelines for Reports of Clinical Trials (Cancer Treat Rep 69:1-3, 1985).

EDITORIAL

For many years the National Cancer Institute has supported the publication of two peer-reviewed journals. Both the *Journal of the National Cancer Institute* (1940) and *Cancer Chemotherapy Reports* (1959) were created to satisfy unmet needs of the scientific community. At the time of its founding, CCR's chief justification was the rapidly growing interest in chemotherapy, as early studies demonstrated that drugs could cure murine tumors and provide meaningful remissions in several different human cancers. In 1976 the renaming of this journal as *Cancer Treatment Reports* recognized the coming of age of clinical oncology and the increasingly multimodal nature of cancer treatment.

Over the past 10 years the contents of CTR have mirrored much of the course of developmental therapeutics. Probably because of its early association with chemotherapy, the journal's image as a forum for early clinical trials of new agents has persisted. Accordingly, the regular journal and its many supplements have retained a focus on chemotherapy and new agent pharmacology, with a liberal scattering of papers relating to multimodality trials, radiotherapy, biological response modifiers, theoretical modeling, and clinical trials methodology. Preclinically, new drug discovery, screening, and pharmacology have dominated.

As times change, however, so do needs. Our field no longer lacks for peer-reviewed journals, sponsored by many professional societies, private foundations, and commercial publishing houses here and abroad. If a federal agency is also to engage in this activity, particularly at a time of budgetary restriction, it should fulfill an essential function that is not being met adequately by the private sector.

What is most striking about contemporary cancer research is the rapid pace of fundamental advances across a very broad front. Increased basic understanding clearly carries enormous promise for prevention and treatment strategies of the future. Unfortunately, but probably inevitably, the frenetic pace of research in so many different directions carries with it the natural tendency for increased compartmentation, just at the time when maximal cross-fertilization has never been so important. Precisely because scientific advance has given developmental therapeutics unprecedented potential, the bench scientist needs an appreciation of the problems posed by clinical cancer. Conversely, clinicians who are unaware of the directions in which the field is moving will be ill-prepared to participate actively as the successive waves of discovery hit the clinic in the years ahead. How many clinical oncologists would have done anything but smile politely at an assertion 10 years ago that the oncologists of the late 1980's would be contemplating the use of antibodies against growth factors and their receptors, immunotoxins, the promotion of terminal differentiation, and rationally based adoptive immunotherapy as viable therapeutic strategies? Finally, to capitalize fully on the present state of intellectual ferment, publication of excellent work must also be fast.

In considering the present needs of the field, therefore, the National Cancer Institute has concluded that the best use of its publications resources is consolidation and reorientation. Pending approval by the federal Office of Management and Budget, we plan the publication of a single journal that will feature new results across the entire spectrum of cancer research—from molecular and cell biology, biochemistry, and carcinogenesis all the way through to phase III clinical trials and epidemiology. The operation of the new journal will feature a peer-review process that is both rigorous and very rapid; the thrust will be to insure excellence of the journal's contents and to minimize the delay between the submission of excellent manuscripts and their publication. To this end, we also plan to publish the journal twice monthly. Editorials, scholarly reviews, letters to the editor, book reviews, and a news section will round out the journal's contents.

The first issue of the new journal is scheduled for January 1988, and we shall soon issue a call for manuscript submissions. The final issue of CTR will appear in December of 1987. In order to phase out operations in an orderly fashion, we shall accept no new submissions after July 15, 1987. We also ask contributors whose papers have received conditional acceptances to return their revised manuscripts within 60 days of their receipt. Revised manuscripts received later than that may or may not be published at the discretion of the Editorial Board.

Those of us who have worked hard with CTR over the years will view its departure from the scene with regret. We are convinced, however, that CTR's current functions will be rapidly subsumed by existing journals with a similar orientation. We hope that cancer scientists and physicians will share our excitement about the potential of a new journal of the kind we have planned and will support it with submission of their best work.

Robert E. Wittes

Contents

Cancer Treatment Reports
Volume 71, Number 3
March 1987

COMMENTARY

229 Reappraisal of Some Dosage Adjustment Guidelines. **A. Sulkes and J. M. Collins.** National Cancer Institute, Bethesda, Maryland

ORIGINAL ARTICLES

235 Transnodal Cancer Chemotherapy for Metastatic Lymph Nodes in Rats. **K. Matsuzaki, Y. Natsuda, H. Kai, and K. Sugimachi.** Kyushu University, Fukuoka, Japan

241 Synergy Between Low-Dose Chemotherapy and Immunotherapy in Mouse L1210 Leukemia. **E. H. Relyveld, B. Bizzini, R. Ophir, and S. Ben-Efraim.** Institut Pasteur, Garches, France

247 Potentiation of Cytotoxicity of Zinostatin Under Acidic Conditions In Vitro and In Vivo. **T. Kuroiwa, S. Taniguchi, K. Aoki, K. Hasuda, and T. Baba.** Kyushu University, Fukuoka, Japan

253 Weekly 5-Fluorouracil Combined With PALA: Toxic and Therapeutic Effects in Colorectal Cancer. **F. M. Muggia, F. J. Camacho, B. H. Kaplan, M. D. Green, E. S. Greenwald, J. C. Wernz, and P. F. Engstrom.** Eastern Cooperative Oncology Group

257 Local Neurotoxicity After Intra-arterial Cisplatin in Head and Neck Cancer. **S. Frustaci, L. Barzan, R. Comoretto, S. Tumulo, G. Lo Re, and S. Monfardini.** Centro di Riferimento Oncologico, Aviano (Pordenone), Italy

261 Thymidylate Synthase Gene Amplification in a Colon Tumor Resistant to Fluoropyrimidine Chemotherapy. **J. L. Clark, S. H. Berger, A. Mittelman, and F. G. Berger.** Roswell Park Memorial Institute, Buffalo, New York

267 Experimental Dacarbazine Antitumor Activity and Skin Toxicity in Relation to Light Exposure and Pharmacologic Antidotes. **R. T. Dorr, D. S. Alberts, J. Einspahr, N. Mason-Liddil, and M. Soble.** The University of Arizona, Tucson

273 Phase I Study of Epirubicin Given on a Weekly Schedule. **R. Snyder, J. Bishop, G. Brodie, W. Burns, A. Coates, J. Levi, D. Raghavan, M. Schwarz, M. Tattersall, D. Thomson, R. Woods, and V. Ziogas.** St. Vincent's Hospital, Fitzroy, Victoria, Australia

277 Second-Line Chemotherapy of Stage III-IV Ovarian Carcinoma: A Randomized Comparison of Melphalan to Melphalan and Hexamethylmelamine in Patients With Persistent Disease After Doxorubicin and Cisplatin. **J. L. Pater, J. A. Carmichael, G. V. Krepart, R. C. Fraser, M. Roy, M. E. Kirk, M. Levitt, L. B. Brown, K. S. Wilson, W. E. Shelley, and A. R. Willan.** Queen's University, Kingston, Ontario, Canada

283 Chemoendocrine Therapy With Prolonged Estrogen Priming in Advanced Breast Cancer: Endocrine Pharmacokinetics and Toxicity. **C. Benz, D. Gandara, B. Miller, T. Drakes, S. Monroe, B. Wilbur, and M. DeGregorio.** Northern California Oncology Group

291 Small Cell Carcinoma of the Lung: Influence of Age on Treatment Outcome. **E. Poplin, B. Thompson, M. Whitacre, and J. Aisner.** University of Maryland Cancer Center, Baltimore

297 Use of Nude Mouse Xenografts as Preclinical Drug Screens: In Vivo Activity of Established Chemotherapeutic Agents Against Melanoma and Ovarian Carcinoma Xenografts. **R. Taetle, F. Rosen, I. Abramson, J. Venditti, and S. Howell.** University of California, San Diego

305 Phase II Trial of Etoposide in APUD Tumors. **D. Kelsen, J. Flore, R. Heelan, E. Cheng, and G. Magill.** Memorial Sloan-Kettering Cancer Center and Cornell University Medical College, New York, New York

BRIEF REPORTS

309 Continuous-Infusion Diaziquone in Acute Myeloid Leukemia: A Southeastern Cancer Study Group Trial. **G. H. Lyman, W. R. Vogler, and M. Raney.** Southeastern Cancer Study Group

311 Phase II Study of Carboplatin in Previously Untreated Patients With Metastatic Small Cell Lung Carcinoma. **R. H. Jacobs, J. D. Bitran, M. Deutsch, P. C. Hoffman, J. Sinkule, S. Purl, and H. M. Golomb.** University of Chicago Medical Center and Michael Reese Medical Center, Chicago, Illinois

313 Percutaneous Hepatic Arterial Infusion of Cisplatin for Metastatic Breast Cancer. **G. Fraschini, G. Fleish-**

man, H.-Y. Yap, C. H. Carrasco, C. Charnsangavej, Y. Z. Patt, and G. N. Hortobagyi. The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston

317 Pharmacokinetic Comparison of Intranasal, Oral, and Intramuscular Metoclopramide in Healthy Volunteers. **M. L. Citron, J. R. Reynolds, J. Kalra, B. G. Kay, K. A. Nathan, N. D. Jaffe, and F. R. Miller.** Long Island Jewish Medical Center, New Hyde Park, New York

CLINICAL TRIALS SUMMARIES

321 Phase II Study of Etoposide in Previously Untreated Squamous Cell Carcinoma of the Head and Neck. **M. A. Cobleigh, J. H. Hill, T. E. Lad, D. E. Shevrin, and E. L. Applebaum.** University of Illinois at Chicago, University of Illinois Hospital, and West Side Veterans Administration Center, Chicago

323 Phase II Study of Aclarubicin in Non-Small Cell Lung Cancer. **J. M. Leiby, W. Hicks, R. W. Roach, and J. A. Neidhart.** The Ohio State University Comprehensive Cancer Center, Columbus

325 Phase II Trial of Esorubicin in Patients With Advanced Melanoma. **S. Frustaci, G. Gasparini, E. Galligioni, U. Tirelli, N. Di Pietro, D. Crivellari, G. Lo Re, and S. Monfardini.** Centro di Riferimento Oncologico, Aviano (Pordenone), Italy

327 Treatment of Recurrent or Metastatic Poorly Differentiated Adenocarcinoma of the Endometrium With Tamoxifen and Medroxyprogesterone Acetate. **R. C. Kline, R. S. Freedman, L. A. Jones, and E. N. Atkinson.** The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston

329 Phase II Trial of Cisplatin and Etoposide in Adenocarcinomas of the Upper Gastrointestinal Tract. **D. P. Kelsen, J. Buckner, A. Einzig, G. Magill, R. Heelan, and V. Vinciguerra.** Memorial Sloan-Kettering Cancer Center, Cornell University Medical College, New York, and the Community Clinical Oncology Program, Manhasset, New York

331 Phase II Studies of PCNU and Bisantrene in Advanced Renal Cell Carcinoma. **P. J. Elson, R. H. Earhart, L. K. Kvols, R. Spiegel, A. M. Keller, M. S. Kies, T. E. Davis, C. Stevens, L. Gumas, and D. L. Trump.** Eastern Cooperative Oncology Group

333 Phase II Clinical Trial of Tricyclic Nucleoside Phosphate for Advanced Colorectal Cancer. **M. J. O'Connell, J. Rubin, R. G. Hahn, L. K. Kvols, and C. G. Moertel.** Mayo Clinic, Rochester, Minnesota

335 Phase II Study of Vinblastine and Bleomycin in Advanced Ovarian Cancer. **R. J. Osborne, V. J. Harvey, M. L. Slevin, J. H. Shepherd, and C. J. Williams.** St. Bartholomew's and Homerton Hospitals, London, England

337 BOOK REVIEW

339 ANNOUNCEMENTS

340 CORRECTION

COMMENTARY

Reappraisal of Some Dosage Adjustment Guidelines[1]

Aaron Sulkes[2,3] and Jerry M. Collins[2,*]

A central tenet of oncologic practice is the desirability of giving each patient the maximum permissible dose of an antineoplastic drug. As summarized recently by Wittes (1), a fundamental assumption in oncology is that more is better, ie, greater efficacy will be produced by higher doses, subject to constraints of host tissue toxicity. Although this principle is firmly imbedded in both research protocols and accepted treatment practices, a special challenge is presented by the patient with impaired liver and/or kidney function. The practical motivation for dosage adjustment is well-understood by all: if the full dose is given to patients with impaired ability to remove the drug from the body, severe and unacceptable toxicity can be expected. Thus, essentially all treatment regimens provide for dosage reduction based upon the status of liver and kidney function tests. Although these dosage reduction schemes are entrenched in everyday oncologic practice, we are not aware of a systematic effort to describe their pharmacologic basis or to validate their usefulness.

In the absence of any other information, a typical empirical approach is to deliver the standard dose of drug or drugs to the patient and then adjust doses on the next cycle, based upon clinical observations of toxicity. This approach can be used to fine-tune decisions based on initial assessment of organ function. It cannot be used as a primary strategy, since patients with impaired elimination may not tolerate the first cycle at the full dose.

The successful use of serum creatinine for dosage adjustments of aminoglycosides (2) and digoxin (3) has encouraged oncologists to adopt the same approach. However, it is worthwhile to keep in mind that there are no similar examples for antineoplastic drugs that are in general use. Recently, Egorin and colleagues (4) have extended the use of serum creatinine and creatinine clearance to the prospective dose-modification of carboplatin, an investigational antineoplastic drug which is primarily excreted unchanged into the urine. They have found that the dose-limiting toxic effect (thrombocytopenia) can be equalized for all patients by titration of dose based upon determination of creatinine clearance (4). Unfortunately, there is no simple test that can be used for titrating the dose of a drug which is actively excreted by the kidneys or metabolized by the liver.

If endogenous markers aren't suitable for most drugs, direct measurements of drug levels are another possible approach. Simple adjustment of dosage would lead to more appropriate levels (5). Alternatively, antidotes can be given for abnormally high levels. Precedent is found in methotrexate plasma-monitoring studies, in which the intensity of leucovorin administration is related to measurements of methotrexate levels in plasma (6). For menogaril, an investigational agent which is primarily eliminated from the body by metabolism, Egorin and colleagues (7) have reported that dosage adjustments can be made on the basis of plasma level measurements. Thus, the dose for an individual can be selected to achieve a specific level of myelosuppression.

An additional consideration occurs for multidrug therapy, since the dose modification factors for each drug are not necessarily identical. For example, the CMF regimen contains three drugs for which the role of renal elimination ranges from dominant (methotrexate) to insignificant (5-fluorouracil) to unclear (cyclophosphamide). A recent publication (8) suggested the use of creatinine clearance to adjust the dosage of both cyclophosphamide and methotrexate, but not 5-fluorouracil. This seems to be a reasonable starting point, but the role of the kidneys must be clarified further to avoid the danger of underdosing for cyclophosphamide. On the other hand, in patients with normal elimination function but low marrow reserve, the use of an equal reduction factor for all myelotoxic agents seems to be appropriate.

SPECIFIC EXAMPLES

We have chosen doxorubicin, cisplatin, and vincristine as examples of drugs for which dosage adjustment

[1] Received June 6, 1986; revised Sept 26, 1986; accepted Oct 6, 1986.

[2] Clinical Pharmacology Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

[3] *Permanent address:* Department of Radiation Therapy and Clinical Oncology, Hadassah Medical Organization, Jerusalem, Israel.

* *Reprint requests to:* Jerry M. Collins, PhD, Clinical Pharmacology Branch, Division of Cancer Treatment, National Cancer Institute, Bldg 10, Rm 6N119, National Institutes of Health, Bethesda MD 20892.

questions must frequently be addressed. For doxorubicin and cisplatin, in particular, there are guidelines which prescribe drastic reductions in dose, based upon liver and kidney function tests, respectively. If the focus is narrowly placed upon avoiding toxicity, these guidelines appear to be successful. However, toxicity could also be avoided by not giving any of these drugs. If there is a strong desire to provide the best available therapy, then the decision-making process must also include a clear consideration of the steep dose-response curves for these drugs and the consequent danger of underdosing.

Doxorubicin

CONVENTIONAL GUIDELINE: If serum bilirubin is between 2 and 3 mg/100 ml and/or SGOT ranges between 100 and 150 U, the dose of doxorubicin should be reduced by 50%. For bilirubin > 3 mg/100 ml and/or SGOT > 150 U, the dose should be decreased by 75%. Patients with serum bilirubin > 5 mg/100 ml should not be given doxorubicin. (Minor differences among institutions exist for specific criteria.)

The dose-response curve for doxorubicin (9,10) should provide a powerful motivation for maximizing the dose given to each patient. However, clinical practice has been strongly influenced by early reports of toxicity and delayed drug elimination in patients with hepatic dysfunction.

Following a dose of 60 mg/m^2, Benjamin et al (11) reported plasma levels of doxorubicin (and especially its metabolites) which were four to five times higher in eight patients with abnormal liver function tests (LFTs) than in patients with normal LFTs. As a group, these eight patients had more severe toxicity from doxorubicin than a group of 79 patients with normal LFTs. Unfortunately, no specific description of the severity of abnormal LFTs was given in this report. These authors then treated an additional nine patients with abnormal LFTs with lower doses of doxorubicin, and the nine patients displayed no unexpected toxicity. However, pharmacology studies were not detailed for these patients. Although all investigators would reduce or withhold doxorubicin doses in patients with severe LFT abnormalities, there is not universal agreement for patients with mildly abnormal LFTs.

Chan et al (12) reported plasma levels of doxorubicin and doxorubicinol in patients with cirrhosis of the liver and hepatoma. When corrected for the lower doses given (10–45 mg/m^2), the profiles of doxorubicin levels in plasma were identical to those obtained in non-hepatoma patients with normal LFTs. However, doxorubicinol exhibited higher peak levels and a prolonged elimination half-life in these hepatoma patients.

In a subsequent study from the same group, Chlebowski et al (13) presented the results from a series of 52 hepatoma patients who were given doxorubicin at a dose of 75 mg/m^2, "unadjusted for hepatic dysfunction." However, the entry criteria included serum bilirubin < 2 mg/100 ml. Nevertheless, some of these patients were given full-dose doxorubicin in the presence of elevated SGOT. Plasma levels were determined for doxorubicin and its metabolites by a specific methodology, ie, thin-film fluorescence scanning. No differences in pharmacokinetics were observed compared with patients with normal transaminase levels. Toxicity was not reported separately for these patients.

Further arguments against indiscriminately decreasing the dose of doxorubicin in patients with abnormal LFTs were provided by the work of Brenner et al (14). No differences in either complete remission rate or toxicity were observed between acute nonlymphocytic leukemia patients with normal LFTs and those with mildly abnormal LFTs (mean serum bilirubin, 2 mg/100 ml; SGOT, 80 U). Patients with more severe abnormalities in LFTs who had doxorubicin dose reduction according to the conventional scheme achieved lower doxorubicin plasma levels, had less toxicity from therapy, and had a response rate similar to that of patients with normal LFTs given full-dose doxorubicin. However, durations of response and survival were decreased in these patients. On this basis, the authors conclude that doxorubicin dose reduction is probably not necessary for mild hepatic dysfunction. Moreover, they were unable to find a convincing quantitative relationship between any liver function test and doxorubicin toxicity or clearance.

In conclusion, it would appear that patients with mildly to moderately abnormal LFTs treated according to the accepted scheme for doxorubicin dosage adjustment are actually *underdosed.* Toxicity may indeed be milder, but effective drug levels are also decreased.

Patients with abnormal LFTs should be given as close to a full dose of doxorubicin as possible. Ideally, doses would be tailored for each case so as to achieve potentially therapeutic but tolerable drug levels and avoid the administration of suboptimal doses by uncritical application of rigid schemes for "dosage adjustment." A prospective study of doxorubicin plasma pharmacokinetics, using specific methodology such as high-performance liquid chromatography, should be undertaken to define target levels for patients receiving this drug. Such a prospective study would also provide the opportunity to observe the extent to which other forms of host deterioration may contribute to increased drug sensitivity in patients with abnormal LFTs. Until the results of such a study are available, it is premature to propose specific guidelines.

Cisplatin

CONVENTIONAL GUIDELINE: Cisplatin should be given only to patients with adequate renal function, defined by a creatinine clearance of at least 50 ml/minute (18,24).

For cisplatin, the conflict is especially sharp between concerns about host organ toxicity and a desire to deliver the largest possible dose. Very early in the clinical testing of cisplatin, it became clear that renal damage would be a major limiting toxic effect (15–17). Indeed, a

critical step in the development of this drug was the improvement of its therapeutic index with the use of hydration, diuresis, and chloruresis (18–20). On the other hand, the steep dose-response relationships that have been shown for ovarian and testicular cancers (21,22) have underscored our interest in maximizing the dose. When patients failed to respond to 50–60 mg/m^2 of cisplatin, they did respond to 100–120 mg/m^2 (23). Patients who have failed at 100 mg/m^2 subsequently responded at 200 mg/m^2 (24).

Contrary to a widely held impression, the kidneys do not provide the main route of cisplatin removal from the body. Inactivation by chemical reaction removes 75% of the administered dose (25). Thus, even if a patient has no renal function, circulating levels of cisplatin will remain similar to levels in patients with normal renal function. Gouyette et al (26) were able to administer five courses of cisplatin to a patient on chronic hemodialysis without inducing any unexpected side effects. The amount of cisplatin removed by dialysis nearly reached the expected renal excretion for patients with normal kidney function. For patients on chronic hemodialysis or anephric patients, this clinical report confirms the pharmacokinetic expectation that dosage adjustment of cisplatin is neither necessary nor desirable.

There is a major difference between the goals of dosage adjustment for doxorubicin and for cisplatin. For doxorubicin, the goal is to protect all host tissues from higher drug levels which would be the consequence of impaired elimination. Ideally, the doxorubicin dosage would be lowered such that the exposure to both normal tissues and tumor would be adjusted to the same range produced by full doses in patients with normal drug elimination.

The motivation for dosage adjustment of cisplatin is to avoid further kidney damage in patients who have inadequate renal function. Unlike the situation for doxorubicin, lowering the cisplatin dose will result in decreased exposure of tumor and normal host tissues, with the expectation of diminished efficacy as well as reduced nonrenal toxicity. Thus, potential benefit versus risk ratios need to be carefully assessed on an individual basis.

Our contention is that abnormal renal function should not automatically obviate the possibility of administering cisplatin at full doses. One must be aware that a major decrease in creatinine clearance following treatment with cisplatin is transient for the vast majority of patients. By the time of the next scheduled course of cisplatin, creatinine clearance rates have either recovered completely (27) or have achieved a level which allows the continued administration of full doses of cisplatin (28,29). Also, for patients with chemoresponsive malignancies in whom renal dysfunction results directly from tumor involvement, cisplatin can be given at the full, planned dose, even 200 mg/m^2 (20,30), since its antitumor effect can "paradoxically" result in improved renal function. In addition, for patients in whom renal function plays a minor role in the decision-making process (such as for anuric patients on chronic hemodialysis), there is no valid reason for giving less than the planned dose of this drug.

There is one subset of patients for whom firm recommendations remain elusive. If renal dysfunction is not due to tumor obstruction and if residual renal function is truly compromised by further doses of cisplatin, neither full-dose cisplatin nor reduced-dose cisplatin will be satisfactory. The clinician must weigh the benefits of full-dose cisplatin against the likelihood of further reduction in renal function, as well as the likelihood of ineffective therapy if renal function is protected by dosage reduction. A potential alternative may emerge from current evaluations of nonnephrotoxic analogs of cisplatin. For carboplatin, in particular, the ability to adjust doses on the basis of renal function has been demonstrated (4).

Vincristine

CONVENTIONAL GUIDELINE: Vincristine is given in single doses of 1.4 mg/m^2 each, and the total dose per administration should not exceed 2 mg (35,40).

Initial clinical trials with vincristine date back close to 25 years. A consistent finding in those early trials was the development of neurotoxicity resulting from repeated administration of the drug. It was readily appreciated that while neurotoxicity was usually related to the cumulative dose, wide interpatient variability existed and some patients were able to tolerate much higher doses of vincristine with mild or no toxicity. In some early trials, single doses up to 2.8 mg/m^2 were given (31,32). In one trial, cumulative doses $\geq$ 30 mg (range, 30–126) were delivered to ten of 50 patients as single doses of $\geq$ 3 mg (33). Nine of these ten patients had no overt neurotoxicity.

Significant interindividual variation has also been shown to occur in the plasma pharmacokinetics of vincristine in man, with an 11-fold range in area under the curve (AUC) values (34). Since neurotoxicity was found to correlate with the plasma AUC (35), susceptibility to the toxic effects of vincristine would differ accordingly from patient to patient. Presumably, this could explain the extremely poor tolerance to vincristine in some patients (36), while others are able to receive massive cumulative doses without clinical impairment (33). We are not aware of any pharmacokinetic basis for assuming that drug exposure is greater in patients with body surface areas exceeding 1.4 m^2. Further pharmacokinetic studies would be facilitated by the development of improved assay methods for vincristine. The present radioimmunoassay methods (37) are convenient for batch analysis of a large number of samples but are nonspecific with respect to metabolites.

With these differences in sensitivity to vincristine among individual patients, applying a rule limiting single doses to a total of 2 mg seems overly rigid and unreasonable. Although this limit of 2 mg is standard in most solid tumor protocols (38–40), this lack of flexibil-

ity is far less prominent in the treatment of lymphomas. There is large published experience (41–43) in lymphoma patients documenting the safety and efficacy of protocols without the 2-mg restriction.

Thus, at the present time, it seems reasonable to recommend starting at the full dose of 1.4 mg/m^2 without the 2-mg total dose restriction. Further administration of the drug should be adjusted as guided by clinical evidence of toxicity. This recommendation is facilitated by the long-appreciated observation that early manifestations of neurotoxicity secondary to the administration of vincristine are reversible upon discontinuation of the drug (36). Prospective monitoring of plasma levels and the incidence of neurotoxicity can be expected to facilitate the development of revised guidelines. In addition, the effect of other risk factors, such as age, can be determined.

CONCLUSION

In general terms, the adjustment of dose for organ function is similar to the concept of dosage adjustment based upon body size, body surface area, or age. The basic strategy is to recognize that a population of patients consists of individuals with a range of capabilities for drug removal from the body. The standard dose of a drug will be based upon experience and will generally be targeted towards the "normal" or average patient. Although the major concern has been directed to patients with reduced capabilities for drug elimination, it should be noted that a patient with above-average capability to remove drug is a candidate for a dose increase above the standard dose.

Although not explicitly stated, the underlying basis for dosage adjustment is inherently pharmacokinetic. Even if plasma levels for a given drug have never been measured, the tacit goal of dose adjustment is to compensate for differences in rates of drug removal from the body. Drug concentration is simply the ratio between drug dose and removal rate. If the removal rate is slower than average, then decreasing the dose will adjust drug exposure back towards average levels.

The overall goal of dosage adjustment should be to reduce the risks of excessive toxicity without compromising a patient's opportunity for therapeutic benefit. Although there is a need to be cautious in the treatment of a patient with impaired elimination organ function, the nature of the steep dose-response curve for most anticancer drugs compels us to consider the problems of underdosing as well. What is needed is a therapeutic strategy which identifies target levels for response as well as toxicity. This goal will not be easily achieved, but the technology exists in many cases to undertake prospective studies towards this definition, which will lead to the development of improved guidelines and individual tailoring of doses for problematic patients.

REFERENCES

1. WITTES RE. Adjuvant chemotherapy—clinical trials and laboratory models. Cancer Treat Rep 70:87–103, 1986.
2. MCHENRY MC, GAVAN TL, GIFFORD RW, JR, ET AL. Gentamicin dosages for renal insufficiency. Adjustments based on endogenous creatinine clearance and serum creatinine concentration. Ann Intern Med 74:192–197, 1971.
3. KOUP JR, JUSKO WJ, ELWOOD CM, ET AL. Digoxin pharmacokinetics: role of renal failure in dosage regimen design. Clin Pharmacol Ther 18:9–21, 1975.
4. EGORIN MJ, VAN ECHO DA, OLMAN EA, ET AL. Prospective validation of a pharmacologically-based dosing scheme for the *cis*-diamminedichloroplatinum(II) analogue diamminecyclobutanedicarboxylatoplatinum. Cancer Res 45:6502–6506, 1985.
5. CHABNER BA. Clinical pharmacokinetics and drug monitoring. *In* Pharmacologic Principles of Cancer Treatment (Chabner BA, ed). Philadelphia, W. B. Saunders, 1982, pp 100–108.
6. STOLLER RG, HANDE KR, JACOBS SA, ET AL. Use of plasma pharmacokinetics to predict and prevent methotrexate toxicity. N Engl J Med 297:630–634, 1977.
7. EGORIN MJ, VAN ECHO DA, WHITACRE MY, ET AL. Human pharmacokinetics, excretion, and metabolism of the anthracycline antibiotic menogaril (7-OMEN, NSC-269148) and their correlation with clinical toxicities. Cancer Res 46:1513–1520, 1986.
8. TAYLOR SG, IV, GELMAN RS, FALKSON G, ET AL. Combination chemotherapy compared to tamoxifen as initial therapy for stage IV breast cancer in elderly women. Ann Intern Med 104:455–461, 1986.
9. O'BRYAN RM, BAKER LH, GOTTLIEB JE, ET AL. Dose-response evaluation of adriamycin in human neoplasia. Cancer 39:1940–1948, 1977.
10. HOLLAND JF. Randomized trials in rare tumors. J Clin Oncol 3:1163–1165, 1985.
11. BENJAMIN RS, WIERNIK PH, and BACHUR NR. Adriamycin chemotherapy—efficacy, safety and pharmacologic basis of an intermittent single high-dosage schedule. Cancer 33:19–27, 1974.
12. CHAN KK, CHLEBOWSKI RT, TONG M, ET AL. Clinical pharmacokinetics of Adriamycin in hepatoma patients with cirrhosis. Cancer Res 40:1263–1268, 1980.
13. CHLEBOWSKI RT, BRZECHWA-ADJUKIEWICZ A, COWDEN A, ET AL. Doxorubicin (75 mg/m^2) for hepatocellular carcinoma: clinical and pharmacokinetic results. Cancer Treat Rep 68:487–491, 1984.
14. BRENNER DE, WIERNIK PH, WESLEY M, ET AL. Acute doxorubicin toxicity. Relationship to pretreatment liver function, response, and pharmacokinetics in patients with acute nonlymphocytic leukemia. Cancer 53:1042–1048, 1984.
15. ROSSOF AH, SLAYTON RE, and PERLIA CP. Preliminary clinical experience with *cis*-diamminedichloroplatinum (NSC-119875 CACP). Cancer 30:1451–1456, 1972.
16. DECONTI RC, TOFTNESS BR, LANGE RC, ET AL. Clinical and pharmacological studies with *cis*-diamminedichloroplatinum. Cancer Res 33:1310–1315, 1973.
17. HIGBY DJ, WALLACE HJ, JR, and HOLLAND JF. *cis*-Diamminedichloroplatinum (NSC-119875): a phase I study. Cancer Chemother Rep 57:459–463, 1973.
18. HAYES DM, CVITKOVIC E, GOLBEY RB, ET AL. High dose cisplatinum diammine dichloride. Amelioration of renal toxicity by mannitol diuresis. Cancer 39:1372–1381, 1977.
19. ETTINGER LJ, DOUGLASS HO, JR, MINDELL ER, ET AL. Adjuvant adriamycin and cisplatin in newly diagnosed, nonmetastatic osteosarcoma of the extremity. J Clin Oncol 4:353–362, 1986.
20. OZOLS RF, CORDEN BJ, JACOB J, ET AL. High-dose cisplatin in hypertonic saline. Ann Intern Med 100:19–24, 1984.
21. OZOLS RF, and YOUNG RC. Chemotherapy of ovarian cancer. Semin Oncol 11:251–263, 1984.
22. SAMSON MF, and RICKIN SE. Dose-response and dose-survival advantage for high versus low-dose cisplatin combined with vinblastine and bleomycin in disseminated testicular cancer. Cancer 53:1029–1035, 1984.
23. BRUCKNER HW, WALLACH R, COHEN CJ, ET AL. High-dose platinum for the treatment of refractory ovarian cancer. Gynecol Oncol 12:64–67, 1981.
24. OZOLS RF, OSTCHEGA Y, MYERS CE, ET AL. High-dose cisplatin in hypertonic saline in refractory ovarian cancer. J Clin Oncol 3:1246–1250, 1985.
25. GORMLEY PE, BULL JM, LEROY AF, ET AL. Kinetics of *cis*-dichlorodiammineplatinum. Clin Pharmacol Ther 25:351–357, 1979.

26. GOUYETTE A, LEMOINE R, ADHEMAR J-P, ET AL. Kinetics of cisplatin in an anuric patient undergoing hemofiltration dialysis. Cancer Treat Rep 65:665–668, 1981.
27. STARK JJ, and HOWELL SB. Nephrotoxicity of *cis*-platinum(II)-dichlorodiammine. Clin Pharmacol Ther 23:461–465, 1978.
28. DENTINO M, LUFT FC, YUM MN, ET AL. Long term effect of *cis*-diamminedichloride platinum (CDDP) on renal function and structure in man. Cancer 41:1274–1281, 1981.
29. VOGELZANG NJ, TORKELSON JL, and KENNEDY BJ. Hypomagnesemia, renal dysfunction and Raynaud's phenomenon in patients treated with cisplatin, vinblastine, and bleomycin. Cancer 56:2765–2770, 1985.
30. OZOLS RF, DEISSEROTH AB, JAVADPOUR N, ET AL. Treatment of poor prognosis nonseminomatous testicular cancer with a "high-dose" platinum combination chemotherapy regimen. Cancer 51: 1803–1807, 1983.
31. COSTA G, HRESHCHYSHYN MM, and HOLLAND JF. Initial clinical studies with vincristine. Cancer Chemother Rep 24:39–44, 1962.
32. HOLLAND JF, SCHARLAU C, GAILANI S, ET AL. Vincristine treatment of advanced cancer: a cooperative study of 392 cases. Cancer Res 33:1258–1264, 1973.
33. WHITELAW DM, COWAN DH, CASSIDY FR, ET AL. Clinical experience with vincristine. Cancer Chemother Rep 30:13–20, 1963.
34. VAN DEN BERG HW, DESAI ZR, WILSON R, ET AL. The pharmacokinetics of vincristine in man: reduced drug clearance associated with raised serum alkaline phosphatase and dose-limited elimination. Cancer Chemother Pharmacol 8:215–219, 1982.
35. DESAI ZR, VAN DEN BERG HW, BRIDGES JM, ET AL. Can severe vincristine neurotoxicity be prevented? Cancer Chemother Pharmacol 8:211–214, 1982.
36. ROSENTHAL S, and KAUFMAN S. Vincristine neurotoxicity. Ann Intern Med 80:733–737, 1974.
37. NELSON RL, DYKE RW, and ROOT MA. Comparative pharmacokinetics of vindesine, vincristine and vinblastine in patients with cancer. Cancer Treat Rev 7:17–24, 1980.
38. ASHENHURST J, BITRAN JD, ROBIN E, ET AL. Melphalan, vincristine, and 5-FU in advanced colonic carcinoma: results of a pilot study. Cancer Treat Rep 65:869–872, 1981.
39. SHIPP SK, MUSS HB, WESTRICK MA, ET AL. Vincristine, doxorubicin and mitomycin (VAM) in patients with advanced breast cancer previously treated with cyclophosphamide, methotrexate and fluorouracil (CMF). Cancer Chemother Pharmacol 11:130–133, 1983.
40. BROOKS BJ, JR, SEIFTER EJ, WALSH TE, ET AL. Pulmonary toxicity with combined modality therapy for limited stage small-cell lung cancer. J Clin Oncol 4:200–209, 1986.
41. DEVITA VT, JR, SERPICK AA, and CARBONE PP. Combination chemotherapy in the treatment of advanced Hodgkin's disease. Ann Intern Med 73:881–895, 1970.
42. SWEET DL, GOLOMB HM, ULTMANN JE, ET AL. Cyclophosphamide, vincristine, methotrexate with leucovorin rescue and cytarabine (COMLA) combination sequential chemotherapy for advanced diffuse histiocytic lymphoma. Ann Intern Med 92:785–790, 1980.
43. SCHEIN PS, DEVITA VT, JR, HUBBARD S, ET AL. Bleomycin, adriamycin, cyclophosphamide, vincristine and prednisone (BACOP) combination chemotherapy in the treatment of advanced diffuse histiocytic lymphoma. Ann Intern Med 85:417–422, 1976.

Transnodal Cancer Chemotherapy for Metastatic Lymph Nodes in Rats[1]

Koichi Matsuzaki,* Yasunori Natsuda, Hidenobu Kai, and Keizo Sugimachi[2,3]

The antitumor effects of transnodal cancer chemotherapy were studied using rat models. After inoculation of AH-66 tumor cells into the iliac lymph nodes (target lymph node), emulsionized bleomycin (BLM emulsion) or BLM water solution was injected into an intact popliteal lymph node and chronological changes in BLM levels in the target lymph node were measured. In the target lymph node of the iliac lymph node, the level of BLM emulsion continuously was > 2 μg/g for 9 hours after the injection, while the level of BLM water solution decreased rapidly. The antitumor effects were quantitatively estimated in the tumor of the target lymph node. The ratio of necrotic area in the BLM-emulsion-treated group was significantly greater than that in the emulsion- or saline-treated groups. Survival rate in rats treated with BLM emulsion was significantly higher than that in control groups and was slightly higher than that in the group given BLM water solution iv. The concentration of BLM in the serum and lung in case of transnodal administration of BLM was much lower than that in rats given an iv administration of BLM water solution. These findings suggest that transnodal administration of BLM emulsion is an effective and safe treatment for metastatic lymph nodes, at least in rats. [Cancer Treat Rep 71:235-239, 1987]

In malignant diseases, the presence of metastatic lymph nodes is one of the most important prognostic factors; therefore, effective therapeutic treatment for metastatic lymph nodes has to be designed. In patients with esophageal carcinoma, lymph node metastasis frequently occurs in the upper mediastinum (1). A complete dissection of these lymph nodes, however, is impossible due to anatomical restrictions; hence there is a high incidence of recurrence in the lymph nodes in the upper mediastinum (2).

We devised a form of intraoperative local adjuvant cancer chemotherapy for metastatic lymph nodes of the mediastinum by giving an anticancer agent, ie, bleomycin (BLM) was injected into the tracheal bifurcation lymph nodes located at the anatomical pivot in the upper mediastinal lymph flow (3). High concentrations of BLM in regional upper mediastinal lymph nodes could be detected using this method.

In the present experiments, we attempted to confirm the anticancer effects of transnodal cancer chemotherapy for metastatic lymph nodes using a new formula of BLM, as well as to establish an experimental system for treating metastatic lymph nodes.

MATERIALS AND METHODS

Experimental Model of Transnodal Cancer Chemotherapy for Metastatic Lymph Nodes

A total of 250 male Donryu rats, weighing 250–300 g, were purchased from Nippon Rat Inc and were provided a commercial pellet food (Nippon Clea Co, Tokyo, Japan) and tap water ad libitum. Established tumor cells, AH-66, derived from a liver tumor in Donryu strain rats (4) were prepared.

AH-66 tumor cells (3×10^6) were inoculated into the iliac lymph node during laparotomy. A newly devised type of BLM emulsion in the form of water in oil (w/o) and stabilized with urographin was used as the anticancer agent (table 1). For transnodal chemotherapy, this BLM emulsion was injected into the popliteal lymph node, under the premise that the injected BLM emulsion would pass through the popliteal lymph node to the target of iliac lymph node bearing tumor, then to the renal lymph node along with flow of lymph. In all the experiments, 0.1 ml of emulsion containing 3 mg of BLM was slowly injected into the popliteal lymph node,

[1] Received Oct 23, 1985; revised Aug 27, 1986; accepted Oct 28, 1986.

[2] Second Department of Surgery, Faculty of Medicine, Kyushu University, Fukuoka, Japan.

[3] We thank M. Ohara, Kyushu University, for critical comments in the manuscript.

* *Reprint requests to:* Koichi Matsuzaki, MD, Oita National Hospital, Yokota 1000-1, Oita City, Oita Prefecture, Japan.

TABLE 1.—Composition of BLM emulsion in w/o*

Lipiodol ultra fluid	0.730 ml
Urographin (60%)	0.152 ml
Buffer (phosphoric acid)	0.036 ml
Tween 80	0.024 ml
Sorbitansesquioleate-15	0.047 ml
Bleomycin water solution	30 mg
Total	1 ml

*Lipiodol and concentrated BLM water solution were stabilized using urographin, and emulsionized in w/o.

using a Hamilton syringe. Tissue adhesive was applied to prevent leakage of the agent from the injected site.

Experiment 1: Measurements of BLM Levels in Regional Lymph Nodes After Transnodal Administration

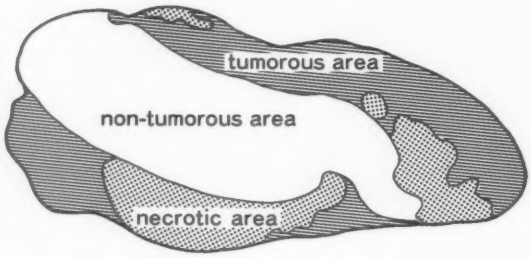
Chronological migration of BLM emulsion from the popliteal lymph node to the proximal lymph nodes in the iliac and renal lymph nodes was observed in both normal and tumor-bearing rats.

At 1, 3, 6, 9, 12, 24, and 48 hours after the injection, the popliteal, iliac, and renal lymph nodes were dissected. BLM levels in the homogenized lymph nodes were measured by bioassay (5). The migration of BLM emulsion from the popliteal to the iliac lymph node was also confirmed with soft x-ray film at 3 hours after the injection.

The levels of BLM in each lymph node after the transnodal administrations were compared. The BLM level in lymph nodes was measured after iv administration of 3 mg of BLM water solution and levels of BLM in serum and lung were also measured.

Experiment 2: Determination of Antitumor Effects of Transnodal Cancer Chemotherapy

AH-66 cells were inoculated into the iliac lymph node, as described above. The next day, 0.1 ml of BLM emulsion containing 3 mg was injected into the popliteal lymph node once daily for 5 days. In the control rats, 0.1 ml of saline or emulsion was injected into the popliteal lymph node, using the same protocol. On Days 8 and 15, the rats were killed and the iliac lymph nodes bearing the tumor were dissected for pathohistological examination. To quantitate the anticancer effects of transnodal cancer chemotherapy, we calculated the necrotic area ratio, as a percentage of the necrotic area versus the entire tumorous area (fig 1). A comparison between effects in the BLM-emulsion-treated group and the control groups was then made.



$$\text{necrotic area ratio} = \frac{\text{necrotic area}}{\text{whole tumorous area}} \times 100\,\%$$

FIGURE 1.—Necrotic area ratio: resected lymph nodes were stained with hematoxylin-eosin and the necrotic area ratio measured.

The survival rate during 60 days after the treatment was compared among the rats in these three groups, ie, the group treated with BLM emulsion and BLM water solution, emulsion and saline, and rats given BLM water solution iv. Statistical analysis was performed using the χ^2 test.

RESULTS

Migration of BLM Levels With Transnodal Cancer Chemotherapy

Deposits of injected contrast medium were observed in the iliac lymph node bearing the tumor, on the soft x-ray film taken 3 days after transnodal chemotherapy.

Chronological changes in levels of BLM in the regional lymph nodes are shown in figure 2. In normal rats with no tumor in the iliac lymph node, the level of BLM in the popliteal lymph node was 80.6 μg/g at 1 hour after injection and decreased gradually to 4.7 μg/g at 48 hours. The iliac lymph node contained 2.12 μg/g of BLM at 1 hour and the levels remained at > 1 μg/g for 9 hours. In the renal lymph node, the BLM level was 1.66 μg/g at 1 hour, was the highest at 3 hours, then decreased to a level < 1 μg/g at 9 hours (fig 2a).

As shown in figure 2b, the rats bearing tumors in the iliac lymph node showed similar levels of BLM in each lymph node. In the tumor-bearing iliac lymph node, the maximal BLM concentration was 3.63 μg/g at 1 hour and the level of about 2 μg/g of BLM was maintained from 3 to 9 hours after the injection. In the renal lymph node, the maximal BLM concentration was 1.56 μg/g at 3 hours after injection, such being comparatively lower than that in normal rats. These findings indicate that the BLM emulsion injected into the popliteal lymph node reached the iliac lymph node and then the proximal renal lymph node.

On the other hand, in case of BLM levels after injection of BLM water solution, the iliac lymph node exhibited higher levels of BLM, 12.2 μg/g at 1 hour, than that in the BLM-emulsion-treated groups. The level of BLM in both lymph nodes decreased rapidly to 0.33 μg/g at 9 hours. The renal lymph node also showed a peak BLM level of 7.8 μg/g at 1 hour and detection was nil at 6 hours (fig 2c). In rats given BLM water solution iv, the BLM level was undetectable in all regional lymph nodes.

The levels of BLM in serum and lung are summarized in table 2. The transnodal-administered group showed a much lower level of BLM, especially in the BLM-emulsion-treated group, as compared with the group administered BLM water solution iv. Here the levels at 1 hour

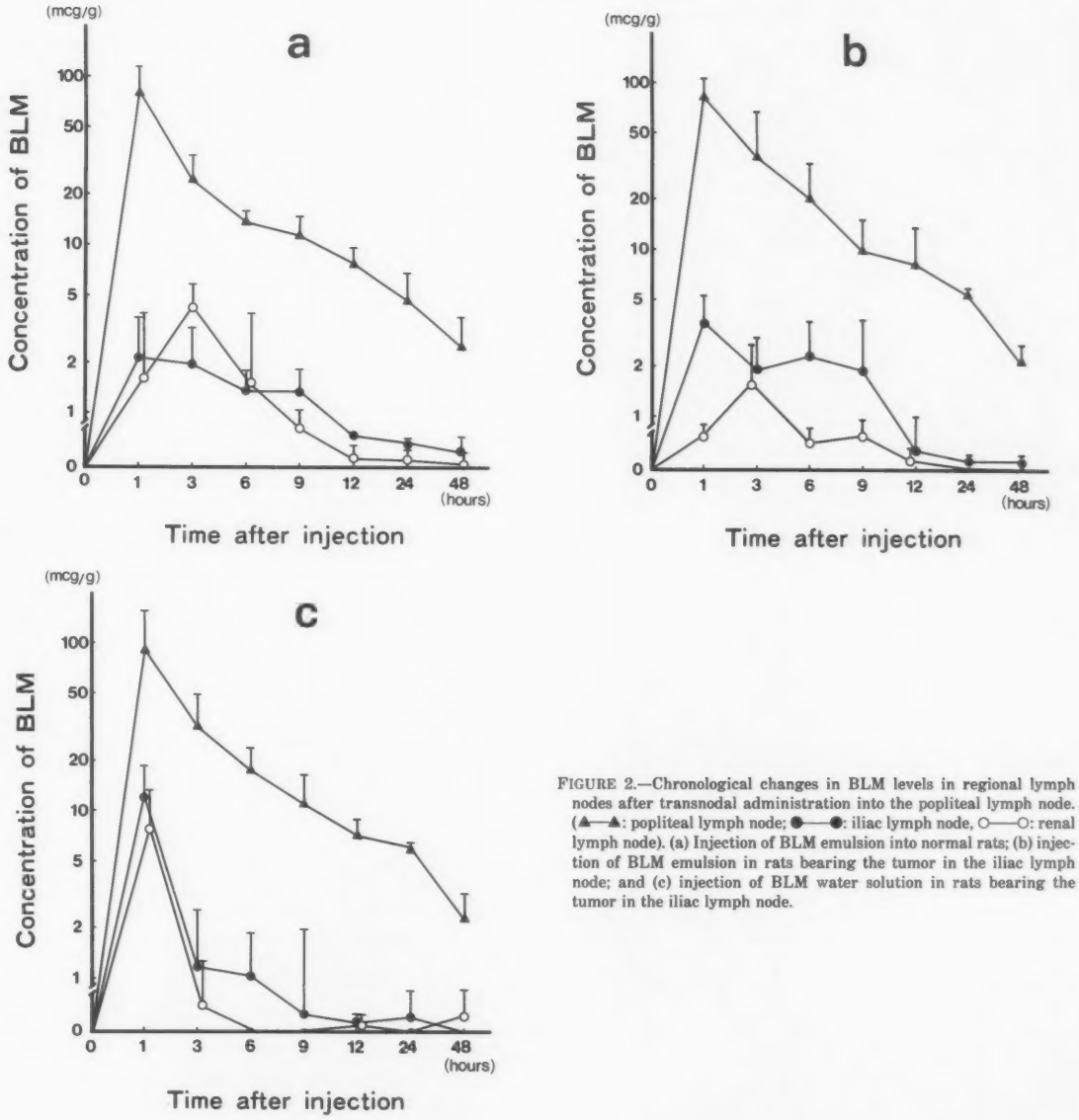


FIGURE 2.—Chronological changes in BLM levels in regional lymph nodes after transnodal administration into the popliteal lymph node. (▲—▲: popliteal lymph node; ●—●: iliac lymph node, ○—○: renal lymph node). (a) Injection of BLM emulsion into normal rats; (b) injection of BLM emulsion in rats bearing the tumor in the iliac lymph node; and (c) injection of BLM water solution in rats bearing the tumor in the iliac lymph node.

were 14.65 μg/ml in the serum and 3.42 μg/g in the lung.

Determination of Antitumor Effects of Transnodal Cancer Chemotherapy

In the control groups given saline or emulsion only, pathohistological examination of the iliac lymph node bearing tumors revealed numerous, viable cell dividing tumor tissues. Otherwise, there were massive necrotic areas at the peripheral region under the capsule of the lymph node, as evidenced in rats treated with transnodal cancer chemotherapy with BLM emulsion. Lipiodol deposits stained with osmium were frequent in the necrotic area.

Comparing the necrotic area ratio in the iliac lymph node at 8 days after the treatment, the rate was 17.3% in the BLM-emulsion-treated group, 5.4% in the emulsion-administered group and 11.3% in the group given only saline. The rate in the BLM-emulsion-treated group was

TABLE 2.—Chronological change in BLM levels in serum and lung after treatment*

BLM level	1 hr	3 hrs	6 hrs	9 hrs	12 hrs	24 hrs	48 hrs
Level in serum (μg/ml)							
Transnodally							
Emulsion	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Water solution	2.56	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Iv							
Water solution	14.65	0.29	0.06	< 0.05	< 0.05	< 0.05	< 0.05
Level in lung (μg/g)							
Transnodally							
Emulsion	< 0.11	< 0.11	< 0.11	< 0.11	< 0.11	< 0.11	< 0.11
Water solution	0.46	0.17	0.13	< 0.11	< 0.11	< 0.11	< 0.11
Iv							
Water solution	3.42	0.25	< 0.11	< 0.11	< 0.11	< 0.11	< 0.11

*The transnodal administration groups showed a much lower level of BLM, especially in the BLM-emulsion-treated group, as compared with the group administered BLM water solution iv at 1 hr.

significantly greater than that in the emulsion-administered group ($P < 0.05$). At 15 days after the treatment, the necrotic area ratio in the BLM-emulsion-treated group was 45%; that is significantly greater than the 24.4% in the emulsion-administered group and 21.7% in the group given only saline ($P < 0.05$).

As shown in figure 3, the survival rate on the 60th day in rats treated with BLM emulsion was 84.6%, a rate significantly higher than the 36.4% in the rats treated with emulsion only and 41.7% in those treated with saline ($P < 0.05$). On the other hand, the survival rate in the group given BLM water solution was 71.4%; that is not significantly higher than in the control groups. The rate in the group treated with BLM water solution iv was 60%, with no statistical significance compared to the group treated with BLM emulsion.

DISCUSSION

In patients with esophageal carcinoma, the 5-year survival rate of those with lymph node metastasis is 15.3%. On the other hand, the rate is 53.8% in those with no lymph node metastasis (1). A complete dissection of regional lymph nodes, however, is impossible because of anatomical restrictions. We reported that transtracheal injection of contrast medium into the subcarinal lymph node made feasible mediastinal lymphography (6). Based on this study, we devised a transnodal administration of anticancer drug via subcarinal lymph nodes located at the anatomical pivot in the upper mediastinal lymph flow (3). The concept of transnodal administration of the drugs was derived from a technique of lymphadenography by intralymph nodal injection of radiopaque materials. Brunn and Engeset (7) reported lymphadenography in which direct injection of contrast medium was given into the lymph node and the regional lymph nodes were visualized in some clinical cases.

Tanigawa (8) noted a high concentration of BLM in regional lymph nodes after the injection of w/o-type BLM emulsion into the appendix of rabbits. The present report may be the first of a direct injection of an anticancer agent into intact lymph nodes in order to treat metastasis in the proximal lymph nodes.

As an experimental model of this approach to therapy, the lymphatic system from the popliteal to retroperitoneal lymph nodes has several advantages, includ-

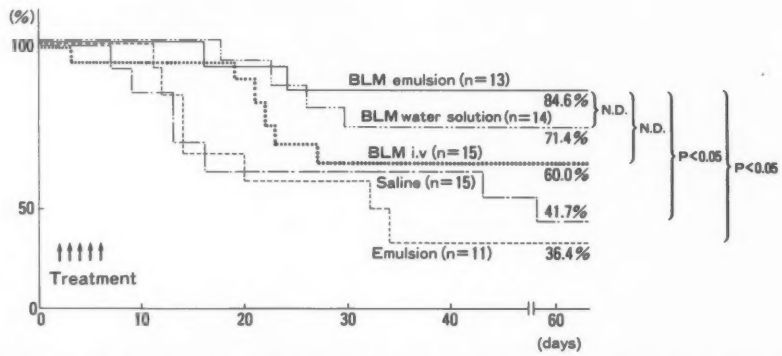


FIGURE 3.—Survival rate: in the BLM-emulsion-treated groups, survival rates were higher than in the control rats ($P < 0.05$) and in the group treated with BLM water solution iv on the 60th day.

ing convenience for injection of the agent, main pathway of lymph flow with few branches (9), and the fact that the three nodes in this pathway (ie, the popliteal, iliac, and renal lymph nodes) can be resected for examination. BLM was used as an antitumor agent as it has a high sensitivity to AH-66 experimental tumor (10) and low cytotoxicity on tissues, thus making feasible a local injection (11).

After injection of BLM emulsion into the popliteal lymph node, the level of BLM in this node gradually decreased from 1 to 48 hours, accompanied by a plateau of BLM levels > 2 μg/g in the proximal lymph node of iliac lymph node bearing the tumor and detection of BLM in the renal lymph node. These observations and the findings on the soft x-ray film indicated that the BLM emulsion injected into the popliteal lymph node migrated through the target node of the iliac lymph node to the most proximal lymph node of renal lymph node. The BLM administered iv was not detected in the target lymph nodes. Therefore, transnodal cancer chemotherapy provides a favorable treatment for metastatic lymph nodes. Transnodal injection of BLM water solution led to rapid decrease in the BLM level in the target lymph node while the plateau of the BLM level > 2 μg/g was observed after the injection of BLM emulsion. Since the anticancer effect of BLM is dose and time dependent (12), this finding suggested that the BLM emulsion is more favorable than the BLM water solution for transnodal cancer chemotherapy.

The antitumor effects of BLM emulsion with transnodal administration were evident. The quantitatively measured necrotic area in the tumors of the target lymph node (iliac) was significantly more obvious in the BLM-emulsion-treated group than in the control groups. Consequently, the survival rate was statistically significant in these rats. As the concentration of BLM in the lung and the serum was low in the transnodal cancer chemotherapy group, in contrast to the higher level of BLM in the iv-administered group, side effects of transnodal cancer chemotherapy are probably minimal.

REFERENCES

1. AKIYAMA H, TSURUMARU M, KAWAMURA T, ET AL. Principles of surgical treatment for carcinoma of the esophagus. Ann Surg 194:438–446, 1981.
2. KASAI M, MORI S, and WATANABE T. Follow-up results after resection of thoracic esophageal carcinoma. World J Surg 2:543–551, 1978.
3. SUGIMACHI K, INOKUCHI K, OKUDAIRA Y, ET AL. Intraoperative local adjuvant cancer chemotherapy for carcinoma of the thoracic esophagus. Jpn J Surg 12:105–110, 1982.
4. FESTING MFW. Inbred strains of rats. Rat News Letter No. 3:13–56, 1978.
5. ISHIZUKA M, TAKAYAMA H, TAKEUCHI T, ET AL. Activity and toxicity of bleomycin. J Antibiot (Tokyo) 20:15–24, 1967.
6. SUGIMACHI K, OKUDAIRA Y, UEO H, ET AL. Transtracheal mediastinal lymphography for visualization of metastatic lymph nodes in carcinoma of the esophagus. Surg Gynecol Obstet 154:34–38, 1982.
7. BRUNN S, and ENGESET A. Lymphography, a new method for the visualization of enlarged lymph nodes and lymphatic vessels. Acta Radiol 45:389–395, 1956.
8. TANIGAWA N. Experimental studies on surgical problems concerning lymphatics III. Surgical chemotherapy against lymph node metastases. Arch Jpn Chir 47:585–593, 1978.
9. RUDOLF H, and STROMBERG MW. Lymphatic system. *In* Anatomy of the Laboratory Rat (Rudolf H, and Stromberg MW, eds). Baltimore, The Williams and Wilkins Company, 1976, pp 112–116.
10. ICHIMURA H. Experimental studies on a screening system of anticancer drugs employing the rat ascites hepatoma spectrum. Jpn J Cancer Chemother 2:605–610, 1975.
11. TAKAHASHI T, UEDA S, KONO K, ET AL. Attempt at local administration of anticancer agents in the form of fat emulsion. Cancer 38:1507–1514, 1976.
12. URANO M, FUKUDA N, and KOIKE S. The effect of bleomycin on survival and tumor growth in a C3H mouse mammary carcinoma. Cancer Res 33:2849–2855, 1973.

Synergy Between Low-Dose Chemotherapy and Immunotherapy in Mouse L1210 Leukemia[1,2]

Edgar H. Relyveld,[3,*] Bernard Bizzini,[3] Rachel Ophir,[4] and Shlomo Ben-Efraim[4,5]

A high dose of 4 mg/kg of daunorubicin (DAU) given in combination with immunostimulation by the P40 immunomodulatory fraction of *Corynebacterium granulosum* and glutaraldehyde (GA)-treated tumor cells coupled with tetanus toxoid (P40 + GA-L1210-Tet) was more effective than DAU alone for treatment of L1210 mouse leukemia. A combination of low doses of DAU (0.0625–0.25 mg/kg) and P40 + GA-L1210-Tet was more effective than either P40 + GA-L1210-Tet or DAU alone. The cured mice were resistant to challenge with a high tumorigenic dose of L1210 tumor cells. A combination of various doses of mitomycin (1–4 mg/kg) with P40 + GA-L1210-Tet was more effective than mitomycin alone and was at least as effective as P40 + GA-L1210-Tet alone. Administration of DAU (0.5–4 mg/kg) to noninoculated C57BL/6 × DBA/2 mice resulted in increase of stimulation in vitro of collected spleen cells by mitogens. [Cancer Treat Rep 71:241–246, 1987]

Combined immunochemotherapy is widely used in experimental tumor systems and in human neoplasia. In previous studies we have shown that chemotherapy with daunorubicin (DAU) or mitomycin (MC) in combination with immunostimulation by the immunomodulatory fraction P40 from *Corynebacterium granulosum* and by chemically modified hemisyngeneic tumor cells was more effective against L1210 mouse leukemia than chemotherapy alone or immunostimulation with P40 alone (1,2).

Use of minimal but still effective amounts of anticancer chemotherapeutic drugs is advantageous because it is less detrimental for normal cells, reduces the risk of side effects such as general immunosuppression and increase of susceptibility to infections, and facilitates development of antitumor immune response. Thus, it has been reported that chemotherapy with a low, nonimmunosuppressive dose of cyclophosphamide (3) or melphalan (4) cured mice bearing large MOPC-315 plasmacytoma tumors because of cooperation between the drug's effect and development of the host's antitumor immune response facilitated by low-dose therapy.

The present work was intended to investigate whether the effective dose for chemotherapy can be lowered when used together with immunostimulation. The results obtained show that low doses of DAU or MC when used together with immunostimulation by the P40 fraction and by L1210 leukemia cells treated with glutaraldehyde (GA) and coupled to tetanus toxoid are more effective or at least as effective as high doses of drug against L1210 leukemia.

MATERIALS AND METHODS

DAU (Specia Laboratories, Paris, France) and MC (Choay Laboratories, Montrouge, France) were used for chemotherapy. The drug solutions were freshly prepared in phosphate-buffered saline (PBS, pH 7.2) and were injected in 0.5 ml ip in four equal quantities for 4 consecutive days starting 1 day after inoculation of tumor cells. The total doses of drugs injected (mg/kg) are given in the Results section and in tables 1–5. The P40 immunomodulatory fraction of *C. granulosum* was prepared as described previously (5). Immunostimulation was performed by combined treatment with P40 and L1210 leukemia cells treated with GA (25% stock solution, Merck-Schuchardt, Federal Republic of Germany) and coupled to purified tetanus toxoid (GA-L1210-Tet cells). Tetanus toxin was produced and purified as described (6). Preparation of GA-L1210-Tet cells was done as de-

[1] Received Jan 2, 1985; revised Oct 16, 1986; accepted Oct 28, 1986.

[2] Supported in part by the Moise and Frida Eskenasy Institute for Cancer Research, Tel-Aviv University, Israel.

[3] Departments of Bacterial Vaccines (*E. H. Relyveld*) and Protein Immunochemistry (*B. Bizziri*), Institut Pasteur, Garches and Paris, France.

[4] Department of Human Microbiology, Sackler School of Medicine, Tel-Aviv University, Israel.

[5] We thank Doctor Chermann, Institut Pasteur, Paris, for the supply of the L1210 tumor line. The technical assistance of Ms. A. M. Catherin and I. Rijks is gratefully acknowledged. We also thank Dr. Ilana Gelernter, Statistical Laboratory, Raymond and Beverly Sackler Faculty of Exact Sciences, School of Mathematical Sciences, Tel-Aviv University, for help and advice in performing statistical calculations.

* *Reprint requests to:* Prof Edgar H. Relyveld, Department of Bacterial Vaccines, Institut Pasteur, 92380 Garches, France.

scribed (2,7). Tetanus toxin was used as a coupling protein for modifying tumor cells because it is available in a highly purified form, is readily converted by GA into toxoid, and the extent of coupling can be accurately determined by using radiolabeled toxin. By using ^{125}I-labeled toxin for coupling, it was found that 0.12 international flocculation units/ml of toxoid was bound to 2×10^7 L1210 tumor cells (approximately 5×10^4 molecules/cell). The quantities per injection in 0.5 ml ip to each mouse were 100 μg of P40 and 5×10^5 GA-L1210-Tet cells. As shown previously (7), the tetanus toxoid coupled to the cells preserved its immunogenic properties because high titers of antitoxin were raised in the immunized mice.

Male C57BL/6 × DBA/2 hybrid mice (5–6 weeks old) used in this study were obtained from Iffa-Credo breeding, l'Arbresle, France. This strain is recommended in the National Cancer Institute conditions of screening (Instruction 14 of the Drug Evaluation Branch-Drug Research and Development Program of the Division of Cancer Treatment).

L1210 leukemia cells were donated by Dr. Chermann, Institut Pasteur, Paris, and maintained by serial ip passages in C57BL/6 × DBA/2 hybrid mice. In experiments, mice were inoculated with 1×10^5 viable L1210 tumor cells ip, a dose which invariably causes formation of ascitic tumors and leads to death in approximately 10 days.

The effectiveness of treatment was evaluated by comparing the median survival times of the groups taken for comparison and by recording the number of cured mice, ie, that of mice remaining alive and free of ascitic tumors at the end of the 60-day observation period after inoculation. The protective effect was expressed by percent increase in life span (% ILS) calculated by comparison with median survival of a corresponding group of inoculated, untreated mice.

Statistical analysis was performed by the log-rank test (8). The difference between two compared groups was considered significant when P was ≤ 0.05. Each experiment included groups of ten mice per group. For calculation of median survival times, an empirical value of 60-day life period was taken for mice remaining alive at the end of the observation period.

The effect of DAU administration on mitogenic stimulation of total spleen cell populations from C57BL/6 × DBA/2 mice was evaluated in cultures incubated with or without mitogen. Spleen cells from mice not treated with DAU were taken as controls. DAU was given in four equal doses in 0.5 ml ip for 4 consecutive days at total doses (mg/kg) as shown in table 5. Spleens were taken 1 day after the last injection of DAU. Single-cell suspensions were prepared by mechanical disruption in Eagle's minimal essential medium (GIBCO, NY), counted microscopically, and viability determined by trypan blue dye exclusion (0.4%). Microcultures were set up in RPMI-1640 medium (GIBCO) supplemented with 10% fetal calf serum, 100 units/ml of penicillin, 100 μg/ml of streptomycin, and 2 nmols/ml of glutamine. Each culture was made in eight parallel samples of 2×10^5 cells/0.1 ml of culture and incubated for 3 days at 37°C in a humidified atmosphere of 5% CO_2 in air. Phytohemagglutinin (PHA) (Wellcome, England, 0.1 μg/0.05 ml/culture) or concanavalin A (ConA; Calbiochem, Switzerland, 0.25 μg/0.05 ml/culture) was added at the beginning of the incubation period. ^{3}H-Thymidine (Nuclear Research Center, Negev, Israel, 1 μCi/0.05 ml/culture) was added for the last 6 hours of incubation.

RESULTS

Chemotherapy With Various Doses of DAU Combined With Immunostimulation

The toxicity of DAU for C57BL/6 × DBA/2 mice was determined in preliminary experiments. DAU was injected in 0.5 ml ip for 4 consecutive days at total doses

TABLE 1.—Effect of immunostimulation alone or combined with a high dose of DAU on the development of L1210 ascitic tumor in C57BL/6 × DBA/2 mice

Serial No.	Group No.	Immunostimulation†	DAU treatment‡	Median survival time (days)	% ILS§	No. of tumor-free mice/total	P value
1*	1	None	None	10		0/30	
	2	P40 + GA-L1210-Tet	None	16	60	5/29	< 0.001¶
	3	None	Yes	15.5	55	0/30	< 0.001¶
	4	P40 + GA-L1210-Tet	Yes	20.5	105	6/30	< 0.001¶
							< 0.01**
							Not significant††

*Mean of 3 experiments, each including 10 animals.

†Immunostimulation was performed by ip injections of P40 immunomodulating fraction of *C. granulosum* (100 μg/injection) and of GA-treated L1210 tumor cells coupled to purified tetanus toxoid (5×10^5 cells/injection) on Days −14 and −7 before inoculation of 1×10^5 viable L1210 tumor cells ip (Day 0) and on Days 2 and 9 after inoculation.

‡Each mouse received 4 ip injections of DAU in 0.5 ml with 1 mg/kg on Days 1–4 after inoculation for a total dose of 4 mg/kg.

§% ILS calculated by comparison with median survival time of control (inoculated untreated mice); for median survival times and ILS calculations a life period of 60 days was considered for the mice remaining alive at the end of the 60-day observation period.

¶P calculated by comparison with the untreated group (Group 1). All P calculations were made by the log-rank test.

**P calculated by comparison between Groups 3 and 4.

††P calculated by comparison between Groups 2 and 4.

TABLE 2.—Effect of immunostimulation combined with chemotherapy with various DAU concentrations on the development of L1210 ascitic tumor in C57BL/6 × DBA/2 mice*

Serial No.	Group No.	Immunostimulation	Drug treatment		Median survival time (days)	% ILS	No. of tumor-free mice/total
			DAU	mg/kg			
2	5	None	None	None	10.0		0/10
	6	P40	None	None	15.5	55	0/10
	7	GA-L1210-Tet	None	None	11.0	10	0/10
	8	P40 + GA-L1210-Tet	None	None	17.0	70	0/10
	9	None	Yes	1	13.0	30	0/10
	10	P40 + GA-L1210-Tet	Yes	1	19.0	90	1/10
	11 and 13†	None	Yes	0.25 or 0.0625	10.5	5	0/20
	12 and 14†	P40 + GA-L1210-Tet	Yes	0.25 or 0.0625	40.0	300	8/19

**See* table 1 for details. Statistical analysis: Groups 11 and 13 vs 12 and 14, $P < 0.001$; Group 8 vs 12 and 14, $P < 0.005$. Differences between Groups 8 vs 10, 9 vs 11, 9 vs 13, 11 vs 13, and 12 vs 14 were not significant.

†Groups 11 and 13 and 12 and 14 were combined for statistical analysis in view of the fact that no significant difference was found between group 11 vs 13 and group 12 vs 14.

ranging from 2 to 12 mg/kg (0.5–3 mg/kg/each injection). A total dose of 4 mg/kg was nontoxic. All mice injected with a total dose of either 8 or 12 mg/kg died (median survival time, 11.8 ± 1.79 and 11.2 ± 1.3 days, respectively). In view of these results, a total dose of 4 mg/kg of DAU was chosen as the high dose of DAU for chemotherapy.

As reported previously (9), treatment with GA-L1210-Tet cells did not induce appreciable protection against a tumorigenic dose of L1210 cells. Treatment with the P40 fraction of *C. granulosum* or with P40 combined with GA-L1210-Tet cells induced a detectable degree of protection against L1210 leukemia (9). Preliminary results (not reported here) have also shown that combination treatment with various doses of DAU (1–4 mg/kg) and with either P40 or GA-L1210-Tet cells did not appreciably improve the treatment with DAU alone. In view of these results the effectiveness of combined chemotherapy and P40 + GA-L1210-Tet cells was investigated.

Treatment with 4 mg/kg of DAU (four equal doses of 1 mg/kg each) led to prolongation of mean survival time from 9.5 days in inoculated untreated mice to 15.6 days. Injection of P40 + GA-L1210-Tet cells alone or together

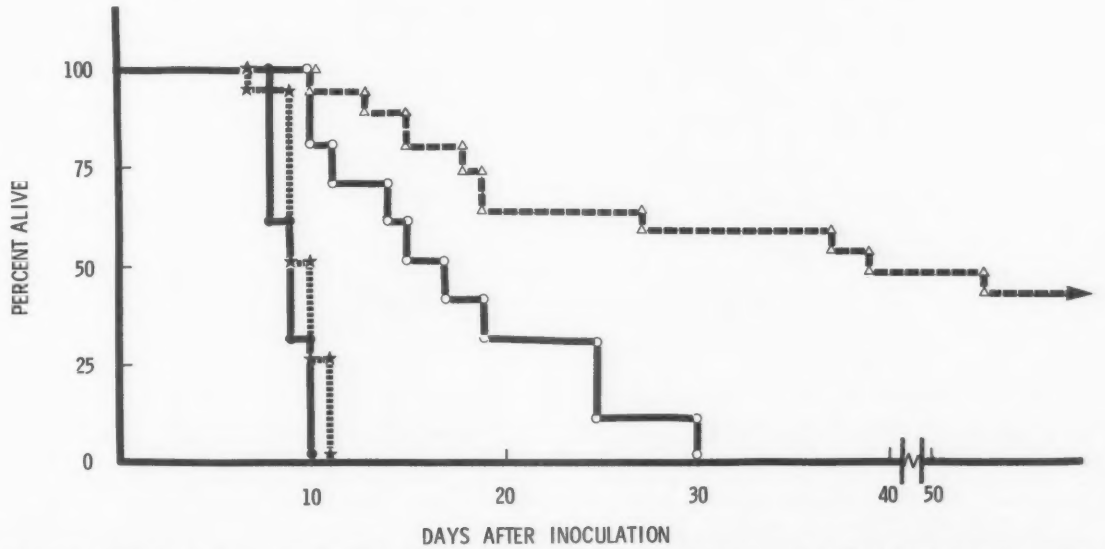


FIGURE 1.—Effect of various treatments on the survival rate of C57BL/6 × DBA/2 mice inoculated ip with 1×10^5 L1210 leukemia cells. ●—● = inoculated untreated mice; 10 mice/group; ★·····★ = inoculated mice treated with either 0.25 or 0.0625 mg/kg total dose of DAU; 20 mice/group; ○—○ = inoculated mice treated with P40 (100 μg ip/injection) and GA-L1210-Tet cells (5×10^5 cells ip/injection) on Days −14 and −7 before inoculation and Days 2 and 9 after inoculation; 10 mice/group; and △—△ = inoculated mice treated with DAU (0.0625 or 0.25 mg/kg) and with P40 + GA-L1210-Tet cells; 19 mice/group.

TABLE 3.—Resistance to challenge of mice cured from L1210 ascitic tumor by combined chemotherapy and immunostimulation treatment*

Serial No.	Group No.	1st inoculation: Treatment	1st inoculation: Median survival time (days)	1st inoculation: No. of tumor-free mice/total	2nd inoculation†: Day after 1st inoculation	2nd inoculation†: No. of tumor-free mice/total
3	15	None	9.0	0/10		
	16	DAU‡	12.0	0/10		
	17	DAU + P40 + GA-L1210-Tet§	29.5	17/40	72	17/17
	18	None¶	9.5			0/4
4	19	None	10.0	0/10		
	20	DAU**	10.5	0/20		
	21	DAU + P40 + GA-L1210-Tet**	29.5	8/20	104	6/8
	22	None¶	10.0			0/10

*See table 1 for details.
†2nd inoculation, 1×10^5 L1210 tumor cells ip.
‡0.5 mg/kg.
§DAU = 0.0625–0.5 mg/kg (4 groups).
¶Control groups inoculated at the same time as the cured mice that received the 2nd inoculation.
**DAU = 0.125 or 0.5 mg/kg (2 groups).

with DAU at a dose of 4 mg/kg led to marked prolongation of survival time of mice inoculated with L1210 tumor cells and some mice remaining alive beyond the observation period of 60 days. The results are shown in table 1.

Injection of lower doses of DAU (total quantities of 0.25 or 1 mg/kg) prolonged the median survival time up to 10–12 days, whereas injection of 0.0625 mg/kg had no effect. Addition of immunostimulation by P40 + GA-L1210-Tet cells to treatment with 0.0625 or 0.25 mg/kg of DAU resulted in prolongation of median survival time by comparison with DAU alone or P40 + GA-L1210-Tet alone. Moreover, some mice treated with low doses of DAU and P40 + GA-L1210-Tet cells remained alive beyond the observation period of 60 days. The results are shown in table 2 and exemplified in figure 1.

The cured mice were highly resistant to challenge with an otherwise tumorigenic dose of L1210 tumor cells (table 3).

Chemotherapy with various doses of MC (1–4 mg/kg) had only a slight therapeutic effect (table 4). A combination of various doses of MC with P40 + GA-L1210-Tet cells was at least as effective as P40 + GA-L1210-Tet alone (table 4).

Effect of DAU on Mitogenic Stimulation

C57BL/6 × DBA/2 mice were injected ip with DAU equally distributed in four injections for 4 consecutive days. The total quantities injected were 0.5, 1.0, 2.0, or 4.0 mg/kg. Spleen cells were harvested 1 day after the last injection of DAU and tested for mitogenic stimulation by ConA and PHA. Stimulation by PHA or ConA was much higher in spleen cell cultures prepared from

TABLE 4.—Effect of immunostimulation combined with chemotherapy with various doses of MC on the development of L1210 ascitic tumor in C57BL/6 × DBA/2 mice*

Serial No.	Group No.	Immunostimulation	Drug treatment: MC	Drug treatment: mg/kg	Median survival time (days)	% ILS	No. of tumor-free mice/total	P† value	P‡ value	P§ value
5¶	23	None	None		11.0		0/30			
	24	P40 + GA-L1210-Tet	None		16.0	45.5	5/29			
	25	None	Yes	4	14.0	27.3	0/30	< 0.001		
	26	P40 + GA-L1210-Tet	Yes	4	24.0	118.2	7/29	Not significant	< 0.001	< 0.001
	27	None	Yes	2	13.0	18.2	0/30	< 0.001	Not significant	
	28	P40 + GA-L1210-Tet	Yes	2	20.0	81.8	7/29	Not significant	< 0.001	< 0.001
	29	None	Yes	1	15.0	36.4	0/30	< 0.005	Not significant	
	30	P40 + GA-L1210-Tet	Yes	1	24.0	118.2	8/28	Not significant	< 0.001	< 0.001

*See table 1 for details.
†*P* calculated by comparison with the group receiving immunostimulation only (Group 24).
‡*P* calculated by comparison with the group receiving the high quantity of MC (Group 25).
§*P* calculated by comparison with the corresponding group receiving MC without immunostimulation: Group 25 vs 26; Group 27 vs 28; and Group 29 vs 30.
¶Mean of 3 experiments.

TABLE 5.—Effect of DAU administration on mitogenic stimulation of spleen cells from C57BL/6 × DBA/2 mice

DAU (mg/kg)*	^{3}H-Thymidine incorporation (cpm ± SD)†		
	Without mitogen	PHA added	ConA added
None	482 ± 46	3703 ± 319	6976 ± 459
0.5	523 ± 43	4045 ± 629	11946 ± 1508
1.0	848 ± 161	3539 ± 590	12161 ± 782
2.0	916 ± 110	4735 ± 1442	12213 ± 1367
4.0	1025 ± 99	8667 ± 499	17825 ± 754

*DAU was injected in 0.5 ml ip for 4 consecutive days in equal doses; mg/kg represents the sum of quantity given in 4 injections; spleen cells were collected 1 day after the last DAU injection.

†Cultures were set in 8 parallel samples of 2×10^5 cells/culture and incubated for 3 days at 37°C; PHA (0.1 μg/culture) or ConA (0.25 μg/culture) was added at the beginning of the incubation period; ^{3}H-thymidine was added for the last 6 hrs of incubation (1 μCi/culture).

mice injected with 4.0 mg/kg of DAU than in spleen cell cultures of normal mice. Stimulation by ConA was also higher in spleen cell cultures prepared from mice injected with lower doses of DAU (0.5–2.0 mg/kg) than in spleen cell cultures from normal mice. The results are shown in table 5.

DISCUSSION

Use of modified tumor cells for immunoprophylaxis or immunotherapy was based on the assumption that certain modifications may increase the immunogenicity of the tumor cells and facilitate induction of antitumor immune response (10,11).

Previous studies have shown that injection of GA-L1210-Tet cells did not induce antitumor protection against the hemisyngeneic L1210 ascitic tumor (9). Injection of P40 immunodulating fraction of *C. granulosum* alone or in association with GA-L1210-Tet cells increased the resistance to mouse L1210 leukemia (1,2,9). Combination chemotherapy with relatively high doses of DAU or MC (4 mg/kg) and immunostimulation with P40 + GA-L1210-Tet cells was more effective than chemotherapy alone. Preliminary results (not shown here) have demonstrated that combined treatment with various doses of DAU (1–4 mg/kg) and either P40 or GA-L1210-Tet cells did not appreciably improve the treatment with DAU alone. In view of these results the aim of the present study was to determine the effectiveness of chemotherapy with a low dose of drug in combination with immunostimulation by P40 + GA-L1210-Tet cells.

The results presented in this study demonstrate that low doses of DAU combined with P40 + GA-L1210-Tet cells were more effective than DAU alone or P40 + GA-L1210-Tet cells only for treatment of mice inoculated with L1210 tumor cells.

It is also of interest that mice cured by combined DAU and P40 + GA-L1210-Tet treatment were highly resistant to an otherwise tumorigenic dose of L1210 cells. In regard to MC treatment, combinations of various doses of MC and P40 + GA-L1210-Tet cells were at least as effective as immunostimulation alone.

The mechanism of synergy between low-dose therapy with DAU and immunostimulation is under study. It is difficult to correlate the finding that low-dose DAU plus immunostimulation is more efficient than high-dose DAU combined with immunostimulation to a possible immunosuppressive effect of the high dose. Thus, injection of low doses of DAU (0.5–2.0 mg/kg) led to an increase of stimulation by ConA of spleen cells taken from the injected animals, but even a high dose of the drug (4 mg/kg) potentiated the mitogenic response to PHA and ConA. It is, of course, still possible that low doses of DAU (0.5 or 1 mg/kg) act selectively on suppressor T cells as was shown to be the case for cyclophosphamide (12–14) and for melphalan (15). Another possibility is that DAU at a high dose of 4 mg/kg induces rapid reduction in the number of L1210 tumor cells, whereas the rate of clearance of tumor cells is slower when lower doses of DAU are used. Slow clearance of tumor cells may facilitate induction of antitumor immune response in conjunction with immunostimulation.

Use of minimal but still effective amounts of chemotherapeutic drugs is advantageous because it reduces the risk of detrimental effect on normal cells, general immunosuppression, and increased susceptibility to infections. It has been reported (16) that conjugation of DAU to dextran comparatively reduces the toxicity of the free drug without affecting its effectiveness in tumor chemotherapy. Our present data indicate that low doses of DAU can be effectively used for chemotherapy when combined with immunostimulation.

REFERENCES

1. RELYVELD EH, BIZZINI B, and BEN-EFRAIM S. Prévention et traitement de la leucémie L1210 de la souris à l'aide de cellules tumorales inactivées et greffées, en association d'un immunomodulateur, la fraction P40, et d'agents antitumoraux. C R Acad Sci [III] 294:571-576, 1982.
2. RELYVELD EH, BIZZINI B, and BEN-EFRAIM S. Prevention and treatment of L1210 mouse leukemia by immunization with xenogenized tumor cells combined with immunostimulation by the P40 fraction of *C. granulosum* and chemotherapy. Int J Immunopharmacol 6:445-450, 1984.
3. HENGST JCD, MOKYR MB, and DRAY S. Cooperation between cyclophosphamide tumoricidal activity and host antitumor immunity in the cure of mice bearing large MOPC-315 tumors. Cancer Res 41:2163-2167, 1981.
4. BEN-EFRAIM S, BOCIAN RC, MOKYR MB, ET AL. Increase in the effectiveness of melphalan therapy with progression of MOPC-315 plasmacytoma tumor growth. Cancer Immunol Immunother 15: 101-107, 1983.
5. BIZZINI B, MARO B, and LALLOUETTE P. Isolement et caracterisation d'une fraction, dite fraction P40, a partir de *Corynebacterium granulosum*. Med Malad Inf 9:408-414, 1978.
6. BIZZINI B, TURPIN A, and RAYNAUD M. Production et purification de la toxine tetanique. Ann Inst Pasteur 116:686-712, 1969.
7. RELYVELD EH, and BEN-EFRAIM S. Preparation of highly immunogenic protein conjugates by direct coupling to glutaraldehyde-treated cells: comparison with commonly used preparations. J Immunol Methods 40:209-217, 1981.
8. PETO R, PIKE MC, ARMITAYE P, ET AL. Design and analysis of

randomized clinical trials requiring prolonged observation of each patient. II. Analysis and examples. Br J Cancer 35:1–39, 1977.

9. RELYVELD EH, BIZZINI B, and BEN-EFRAIM S. Effet protecteur de la fraction P40 isolée de *C. granulosum* dans la leucémie L1210 de la souris. C R Acad Sci [III] 293:39–42, 1981.
10. KOBAYASHI H. Xenogenization of tumor cells. *In* Gann Monograph on Cancer Research, No. 21, Cancer Immunotherapy and Its Immunological Basis (Yamamura Y, Kitagawa M, and Azuma I, eds). Tokyo, Scientific Societies Press, 1978, pp 21–35.
11. WOODRUFF MFA. Modified tumor cells and cross-reacting antigen. *In* The Interaction of Cancer and Host: Its Therapeutic Significance (Woodruff MFA, ed). New York, Grune and Stratton, 1980, pp 227–228.
12. DIAMANTSTEIN T, KLOS M, HAHN H, ET AL. Direct in vitro evidence for different susceptibilities of antigen-primed T cell regulating humoral and cell-mediated immune responses to sheep erythrocytes: a possible explanation for the inverse action of cyclophosphamide on humoral and cell-mediated immune responses. J Immunol 126:1717–1719, 1981.
13. OZER HJ, COWENS J, COLVIN M, ET AL. In vitro effects of 4-hydroxyperoxycyclophosphamide on human immuno-regulatory T subset function. I. Selective effects on lymphocyte function in T-B cell collaboration. J Exp Med 155:276–290, 1982.
14. KLAJMAN A, DRUCKER I, MANOR J, ET AL. In vitro effects of 4-hydroxyperoxycyclophosphamide on concanavalin A induced suppressor T cells. Cancer Immunol Immunother 18:113–117, 1984.
15. BEN-EFRAIM S, KOMLOS L, NOTMANN J, ET AL. In vitro selective effect of melphalan on human T-cell populations. Cancer Immunol Immunother 19:53–56, 1985.
16. LEVI-SCHAFFER F, BERNSTEIN A, MESHORER A, ET AL. Reduced toxicity of daunorubicin by conjugation to dextran. Cancer Treat Rep 66:107–114, 1982.

Potentiation of Cytotoxicity of Zinostatin Under Acidic Conditions In Vitro and In Vivo[1,2]

Toshiro Kuroiwa, Shun'ichiro Taniguchi, Ken Aoki, Keitaro Hasuda, and Tsuneo Baba[3,4,*]

The cytotoxicity and antitumor effects of zinostatin (NCS) were examined under various conditions of pH using cultured HeLa cells and a transplantable tumor line of WKA rats. The cytotoxicity of NCS against HeLa cells was highly dependent on the pH at ranges of 6–8. When HeLa cells were treated with 0.075 μg/ml of NCS, the survival rate was about 1% at pH 6.0, while it was near 90% at pH 7.4 and pH 8.0. Even at pH 6.5–6.75, the survival rate was about 50% lower than that seen with pH 7.4 and pH 8.0. The ratio of the dose of NCS for a 90% cell mortality at pH 6.0 to that at pH 7.4 was less than one-seventh. Antitumor effects were assessed by local intra-arterial infusion against tumors 4 or 9 days after inoculation of 10^6 RBT-1 tumor cells into the thigh of rats. When NCS (0.7 mg/kg) in phosphate-buffered saline (PBS) (pH 7.4) or 5 mM lactate (about pH 3.0) was administered, the ratio of average tumor weight about 2 weeks after treatment (tumor weight of the rats given NCS in PBS/tumor weight of the rats given NCS in lactate) was 3.2 or 1.7 for the rats treated 4 or 9 days after tumor inoculation, respectively. Lactate or PBS alone had no effect on the growth of tumor. After treatment there was no difference in body weight change between the group treated with NCS in lactate and the group treated with NCS in PBS. We conclude that the cytotoxicity of NCS was potentiated under acidic conditions, both in vitro and in vivo. [Cancer Treat Rep 71:247-251, 1987]

The ultimate objective of cancer chemotherapy is the total lethality of malignant cells, with no untoward effects on normal cells. One approach to making related assessments is the higher glycolysis of cancer cells with increased production of lactic acid, hence an acidic condition in cancer tissues compared to normal tissues (1,2). Accordingly, an anticancer drug, the action of which is potentiated under acidic conditions, may have selective antitumor effects (3–7). The pH dependency of carbazilquinone is an example of the selective attack of cancer cells (8–10).

We now report that the cytotoxicity and antitumor effects of zinostatin (NCS) are also enhanced under acidic conditions, both in vitro and in vivo.

MATERIALS AND METHODS

Chemicals

Original NCS solution (liquid form, Lot K 4579), donated by Kayaku Co, Ltd, Tokyo, Japan, was used after dilution with various vehicles. Lactate was obtained from Wako Pure Chemical Industries, Ltd, Osaka, Japan, and was used as a vehicle for chemotherapy experiments after dilution with 0.9% NaCl at 5 mM.

Animals

Wistar King A (WKA) female rats were obtained from the Animal Center of Kyushu University and maintained on a standard laboratory diet and tap water ad libitum. Rats weighing 140–210 g at 6–8 weeks of age were used for experiments.

Assay of Cytotoxicity of NCS to HeLa Cells

HeLa cells were cultured at 37°C in Eagle's minimal essential medium (Nissui Seiyaku Co, Tokyo, Japan) supplemented with 10% newborn calf serum (GIBCO) or 10% fetal bovine serum (GIBCO) in a humidified atmosphere of 10% CO_2. For the assay of NCS cytotoxicity, log-phase cells harvested with 0.25% trypsin were suspended in growth medium and plated in 50-mm plastic dishes at 2×10^2, 2×10^3, and 2×10^4 cells/plate. The

[1] Received Mar 17, 1986; revised Oct 14, 1986; accepted Oct 28, 1986.
[2] Supported in part by a Grant-in-Aid for Cancer Research from the Ministry of Education, Science and Culture, Japan, and from the Cancer Association of Fukuoka Prefecture, Japan.
[3] Department of Experimental Cell Research, Medical Institute of Bioregulation, Kyushu University 69, Fukuoka, Japan.
[4] We thank M. Ohara for valuable comments on the manuscript.
* *Reprint requests to:* Tsuneo Baba, MD, Department of Experimental Cell Research, Medical Institute of Bioregulation, Kyushu University 69, Maidashi 3-1-1, Higashi-ku, Fukuoka 812, Japan.

plated cells were cultured for 6 hours at 37°C. After removal of the medium, 2.5 ml of phosphate-buffered saline (PBS) at various pHs, with or without NCS, was added to each plate, followed by incubation for 30 minutes at 37°C. The treated cells were washed with 5 ml of cold PBS (pH 7.4) and 5 ml of fresh growth medium was provided. All experiments were repeated three times and triplicate cultures were used for each point. These cells were cultured for 8 days at 37°C, with a change of medium once on Day 5. On Day 8, the number of colonies was counted after staining with Giemsa. The survival rate was calculated as follows: number of colonies treated with NCS in each pH/number of colonies treated with PBS alone at each pH.

Tumor

A transitional cell carcinoma of the bladder (RBT-1) induced in WKA rats with *N*-butyl-*N*-(4-hydroxybutyl)-nitrosamine was maintained by serial transplantation into the hind limb of syngeneic WKA rats every 10–12 days. The tumor was excised, minced, suspended in PBS (pH 7.4), and passed through a metal sieve to obtain a single-cell suspension. After a trypan blue exclusion test, 10^6 viable cells/rat were injected im into the right hind limb of each WKA rat.

Chemotherapy Experiments

Chemotherapy experiments were carried out on Days 4 and 9 after tumor inoculation. The initial tumor size was equivocal on Day 4; on the other hand, it was the tip of the little finger size (about 2.6 cm^3) on Day 9. All rats were anesthetized with ether. In the group given an intrafemoral artery infusion of 0.7 mg/kg of NCS (0.56 × LD50), the original NCS solution (1 mg/ml) was diluted to 0.14 mg/ml with cold PBS (pH 7.4) or cold 5 mM lactate (pH 3.0) 1–2 minutes prior to administration and kept on ice and shielded from light until use. NCS solutions diluted with each vehicle were injected at a flow rate of 1.2 ml/minute through a fine tapered polyethylene catheter inserted into the femoral artery under an operation microscope (Konan Camera, R and I, K-280, Nishinomiya, Japan) using an infusion pump (Harvard Apparatus, Model 975E, Boston, MA). After giving NCS, the catheter was removed and the femoral artery was ligated to avoid bleeding. As for controls, intra-arterial administrations of 5 mM lactate (pH 3.0) or PBS (pH 7.4) were given in the same volume as for the groups given NCS. We added two more groups as controls, ie, untreated and those subjected to femoral artery ligation alone.

Evaluation of Antitumor Effects

Rats treated on Day 4 or 9 after tumor inoculation were killed 17 or 14 days after treatment, respectively, and tumor weights were measured. The antitumor effect of NCS in each vehicle was evaluated by the following calculation: tumor weight infused with NCS/tumor weight infused with the vehicle alone.

Evaluation of General Toxicity

The tumor-bearing rats were weighed on various days after treatment.

RESULTS

Cytotoxicity of NCS to HeLa Cells

Figure 1 shows the survival curve of HeLa cells treated with 0.075 μg/ml of NCS dissolved in PBS of various pHs. The cytotoxicity of NCS was obviously dependent on the pH and increased with lowering of the pH. While the cells were hardly damaged by NCS at pHs > 7.4, the survival was near 0.01 at pH 6.0. Under acidic conditions at about pH 6.5, the survival rate was 30%. The survival curve was compared between pH 6.0 and pH 7.4 by changing the doses of NCS (fig 2). A 90% lethality was seen with 0.04 μg/ml at pH 6.0 and 0.29 μg/ml at pH 7.4, respectively. The slope of the curve at pH 6.0 was steeper than at pH 7.4, and the shoulder which was observed at pH 7.4 in a range of low doses of NCS disappeared at pH 6.0.

Treatment with PBS alone had no effect on the survival of HeLa cells over a range of pH 6.0–8.0 (data not shown).

Antitumor Effects of Chemotherapy Experiments

Tables 1 and 2 show antitumor effects of various treatments given on Day 4 (table 1) or Day 9 (table 2) after tumor inoculation. The average tumor weight in

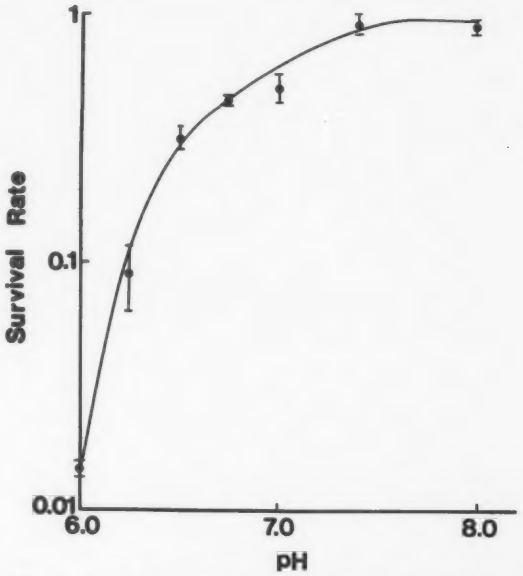


FIGURE 1.—Cytotoxicity of NCS at 0.075 μg/ml to HeLa cells under various conditions of pH. Each point indicates the mean of triplicate cultures ($n = 3$). Bars = SE.

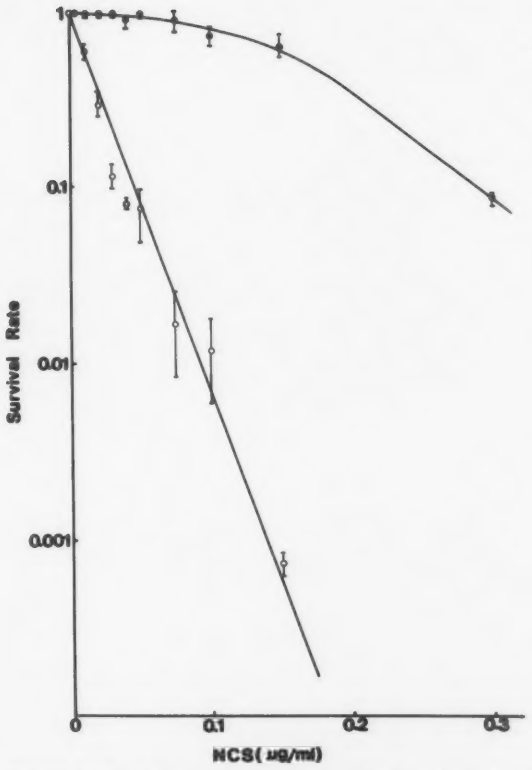


FIGURE 2.—Survival rate of HeLa cells treated with various doses of NCS at pH 7.4 (●) or pH 6.0 (○) for 30 mins. Each point indicates the mean of triplicate cultures ($n = 3$). Bars = SE.

rats given NCS in lactate was minimal and significantly less than that of rats given NCS in PBS (pH 7.4). The tumor weight in rats with femoral arterial ligation alone was half that of the untreated control. There was no statistically significant difference in tumor weight among the groups given intra-arterial infusion of 5 mM lactate, pH 7.4 PBS, or femoral arterial ligation alone.

TABLE 1.—Potentiation of anticancer effects of NCS under acidic conditions on Day 4

Treatment*	No. of rats	Tumor weight in g (mean ± SEM)	P value†
(*a*) Untreated control	10	20.10 ± 1.50	
(*b*) 5 mM lactate	10	9.92 ± 1.04	< 0.01
(*c*) NCS‡ in lactate	10	1.91 ± 0.25	< 0.01
(*d*) PBS	10	10.18 ± 1.00	< 0.01
(*e*) NCS in PBS	10	6.15 ± 1.04	< 0.01
(*f*) Femoral artery ligation	10	9.90 ± 0.74	< 0.01

*Treatments were given on Day 4 after inoculation of 10^6 viable RBT-1 cells into the hind limb.

†Probability was calculated by Wilcoxon test. *P* values for *c* vs *e* = < 0.01; and *b* vs *f* and *d* vs *f* = not significant.

‡0.7 mg/kg of NCS was given.

TABLE 2.—Potentiation of anticancer effects of NCS under acidic conditions on Day 9

Treatment*	No. of rats	Tumor weight in g (mean ± SEM)	P value†
(*a*) Untreated control	10	21.54 ± 1.55	
(*b*) 5 mM lactate	10	12.88 ± 1.50	< 0.01
(*c*) NCS‡ in lactate	10	5.43 ± 0.62	< 0.01
(*d*) PBS	10	11.28 ± 1.39	< 0.01
(*e*) NCS in PBS	10	9.41 ± 1.16	< 0.01
(*f*) Femoral artery ligation	10	11.24 ± 1.17	< 0.01

*Treatments were given on Day 9 after inoculation of 10^6 viable RBT-1 cells into the hind limb.

†Probability was calculated by Wilcoxon test. *P* values for *c* vs *e* = < 0.05; and *b* vs *f* and *d* vs *f* = not significant.

‡0.7 mg/kg of NCS was given.

Although there was no statistically significant difference in tumor weight between the groups given the vehicle alone, the average weight of the tumor in rats treated with NCS in lactate or PBS was divided by that in rats treated with lactate or PBS alone, respectively. Hence, a strict comparison could be made of the net antitumor effects of NCS:

$$Tc/Tb(= 0.19) < Te/Td(= 0.60) \text{ (table 1), and}$$
$$Tc/Tb(= 0.42) < Te/Td(= 0.83) \text{ (table 2),}$$

where Tc = average weight of tumor in rats treated with NCS, using lactate as a vehicle; Tb = average weight of tumor in rats treated with lactate alone; Te = average weight of tumor in rats treated with NCS, using PBS (pH 7.4) as a vehicle; and Td = average weight of tumor in rats treated with PBS (pH 7.4) alone.

The net antitumor effect of NCS in lactate was enhanced compared to that in PBS, irrespective of the initial size of the tumor at the time of treatment.

Side Effects With Chemotherapy

The transient loss of body weight in rats treated with NCS was maximum on Day 1 and the initial levels were reverted to within 5 days. However, there was no significant difference in body weight change between the groups given 0.7 mg/kg of NCS in lactate and in PBS (pH 7.4) (fig 3).

DISCUSSION

According to our in vitro study, the cytotoxicity of NCS against HeLa cells was highly pH-dependent and was potentiated under conditions of a low pH (fig 1). The survival curve at pH 6.0 was steeper than that at pH 7.4, and the shoulder presented at pH 7.4 was removed at pH 6.0. This may indicate that not only the reactivity of NCS with cellular targets but also the number of targets is affected by change of pH.

To ascertain whether NCS would have a more potent cytocidal effect in vivo at a pH lower than the normal of 7.4, we carried out chemotherapy experiments on tumors in the hind limb of rats. Because NCS in the blood

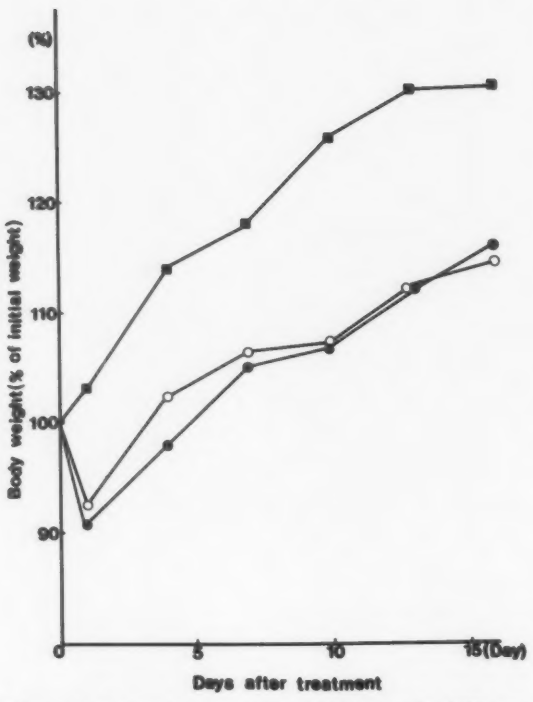


FIGURE 3.—Changes in average body weight of rats after NCS treatments. ■ = untreated control; ○ = 0.7 mg/kg of NCS in 5 mM lactate; and ● = 0.7 mg/kg of NCS in PBS (pH 7.4). Each point is the mean of 10 rats.

is rapidly inactivated (half-life is about 7 minutes) (11,12), we administered NCS locally via intra-arterial infusion. The tumoricidal effects of NCS were also increased under an acidic condition, where 5 mM lactate was used as the vehicle for NCS, compared to cases when the conventional vehicle, PBS (pH 7.4), was used. When NCS was administered iv, little therapeutic effect was gained at any dose (data not shown).

NCS in a vehicle free of its targets, such as protein and nucleic acid, retains > 90% of its activity over the pH range from 2.0 to 8.0 when the incubation time at 37°C is within a few hours (13,14). To eliminate the inactivation of NCS, however, we made up NCS solutions in 5 mM lactate (pH 3.0) or PBS (pH 7.4) just prior to intra-arterial infusion.

We gave glucose to rats systemically prior to the administration of NCS expecting a specific lowering of the pH in tumor tissues. The antitumor action of NCS was, however, little activated by glucose pretreatment. The reasons for this remain to be clarified: the best timing or dose of glucose treatment might not have been used. When we gave 5 mM lactate with or without NCS locally through the femoral artery, necrosis or ulcer formation in normal tissue of the treated limb did not occur (data not shown). Therefore, it is likely that NCS in lactate selectively attacks tumor cells in our local chemotherapy system and that 5 mM lactate, at the doses given, induced no harmful effects on normal tissues.

The potentiation of NCS under acidic conditions might be caused by: (*a*) increase of NCS uptake into cells; (*b*) increase in the reactivity of NCS with biomolecules, such as DNA and protein; and (*c*) decrease in the number of cellular targets, probably caused by inactivation of repair systems, and so on. Direct proof has not been obtained because labeled NCS was not available. Nonprotein chromophore of the NCS possesses DNA strand-breaking activity and the ability to inhibit DNA synthesis (15), while it rapidly loses biochemical activity > pH 4.8 at 37°C (16). The significance and the contribution of the pH-dependent stability of the chromophore to the pH-dependency of NCS on cytotoxicity is now being studied by measuring the proportion of chromophore in the NCS solution.

CONCLUSION

The cytotoxicity of NCS was highly pH-dependent and enhanced under acidic conditions in vitro. Based on this finding, local chemotherapy experiments using NCS for a solid tumor grown in rats were done by changing the acidity of the vehicle. Intra-arterial infusion of NCS in an acidic vehicle (5 mM lactate) also had a greater antitumor effect, with no increase of general toxicity over that seen with the conventional vehicle (PBS of pH 7.4).

REFERENCES

1. KAHLER H, and ROBERTSON WB. Hydrogen-ion concentration of normal liver and hepatic tumors. J Natl Cancer Inst 3:495-501, 1943.
2. WARBURG O, POSENER K, and NAGELEIN E. Über den Stoffwechsel der Carzinomzelle. Biochem Ztschr 152:309-344, 1924.
3. ROSS WCJ. The chemistry of cytotoxic alkylating agents. Adv Cancer Res 1:397-449, 1953.
4. BROPHY GT, and SLADEK NE. Influence of pH on the cytotoxic activity of chlorambucil. Biochem Pharmacol 32:79-84, 1983.
5. HULT RL, and LARSON RE. Dissociation of 5-fluorouracil uptake from intracellular pH in Walker 256 carcinosarcoma. Cancer Treat Rep 60:867-873, 1976.
6. KENNEDY KA, MCGURL JD, LEONDARIDIS L, ET AL. pH dependence of mitomycin C-induced cross-linking activity in EMT6 tumor cells. Cancer Res 45:3541-3547, 1985.
7. MOORE CW, and VOSSLER DA. Influence of pH and length of post-treatment incubation on bleomycin-induced DNA damage. Biochim Biophys Acta 610:425-429, 1980.
8. KIMURA NT, KANEMATSU T, and BABA T. Potentiation of anticancer drug effect by cancer cell glycolysis: an in vitro experiment. Gann 65:513-522, 1974.
9. BABA T, AOKI K, KIMURA NT, ET AL. Potentiation of anticancer effect of carboquone in vivo by glucose pretreatment. Gann 70:255-258, 1979.
10. TANIGUCHI S, KIMURA NT, and BABA T. Effects of pH on cytotoxicity of carboquone. Jpn J Pharmacol 31:243-247, 1981.
11. FUJITA H, NAKAYAMA N, SAWABE T, ET AL. In vivo distribution and inactivation of neocarzinostatin. Jpn J Antibiot 23:471-478, 1970.
12. MAEDA H. Neocarzinostatin in cancer chemotherapy (review). Anticancer Res 1:175-186, 1981.

13. ISHIDA N, MIYAZAKI K, KUMAGAI K, ET AL. Neocarzinostatin, an antitumor antibiotic of high molecular weight. J Antibiot 18:68–76, 1965.
14. KOHNO M, ISHII F, HANEDA I, ET AL. Studies on the stability of antitumor protein, neocarzinostatin. II. Stability of injection of neocarzinostatin. Jpn J Antibiot 27:715–724, 1974.
15. KAPPEN LS, NAPIER MA, and GOLDBERG IH. Roles of chromophore and apo-protein in neocarzinostatin action. Proc Natl Acad Sci USA 77:1970–1974, 1980.
16. KAPPEN LS, and GOLDBERG IH. Stabilization of neocarzinostatin nonprotein chromophore activity by interaction with apoprotein and with HeLa cells. Biochemistry 19:4786–4790, 1980.

Weekly 5-Fluorouracil Combined With PALA: Toxic and Therapeutic Effects in Colorectal Cancer[1,2]

Franco M. Muggia,[3,*] Fernando J. Camacho,[4] Barry H. Kaplan,[4] Michael D. Green,[3] Edward S. Greenwald,[4] James C. Wernz,[3] and Paul F. Engstrom[5]

A total of 51 patients (eight previously treated) received PALA added to 5-fluorouracil (5FU) given weekly. After 32 patients, the PALA schedule was changed from every other week to weekly, 24 hours preceding 5FU in accordance with preclinical leads (*see* text). Both schedules were associated with moderately severe toxic effects related primarily to PALA (skin rash) or to the combined effects of both drugs (diarrhea, vomiting, conjunctivitis, and neurotoxicity). Overall nine partial responses were observed, including three in patients previously treated with 5FU. However, future studies with this combination utilizing the current or other previously published schedules are not warranted in colorectal cancer. Since toxicity is a prominent impediment, the possibility of therapeutic synergy may perhaps be explored at drastically reduced doses of PALA, combined with other modulating measures. [Cancer Treat Rep 71:253-256, 1987]

The limited effectiveness of 5-fluorouracil (5FU) has stimulated clinical and laboratory research into methods to enhance its action against colorectal cancer. *N*-Phosphonacetyl-L-aspartic acid (PALA) has held interest as a modulator of 5FU action: it is a transition-state inhibitor of the enzyme aspartate transcarbamylase involved in the second step of de novo pyrimidine biosynthesis (1,2), and it has undergone detailed preclinical evaluation and clinical phase I and II trials (3-16). PALA leads to a reduction in intracellular uridine pools (17) and its effects may be reversed by nucleoside salvage. It has therefore been considered as a candidate for synergistic interactions with 5FU—by potentiating 5FU effects either through inhibition of DNA synthesis or through incorporation into RNA. In Sarcoma 180 cells, PALA exposure led to a decline in dUMP levels and increased FdUMP relative to 5FU alone (18), which led to increased inhibition of thymidylate synthetase. In several other systems, synergistic cytotoxicity was related to increased FUTP levels and enhanced incorporation of tritiated FU metabolites into RNA, which follows uridine depletion (19-21). In addition, PALA results in increased intracellular phosphoribosyl pyrophosphate (PRPP), presumably through its reduced utilization, and this, in turn, favors formation of FdUMP (20-22).

Demonstration of therapeutic enhancement in tumor-bearing mice (19,23) stimulated early clinical trials of 5FU-PALA combinations (24-30). Although such trials evolved with much expectation and led to early encouraging observations, prominent toxic effects were also encountered. These included primarily either dose-limiting mucositis and diarrhea or incapacitating neurotoxicity. The current trial, performed as a pilot study of the Eastern Cooperative Oncology Group (ECOG), differed from prior trials in attempting to utilize full therapeutic doses of 5FU by the weekly schedule while PALA was given biweekly (Schedule I). Subsequently, in order to more closely mimic optimal experimental conditions which suggested a time sequence for synergy (18,19), 5FU was to be given weekly 24 hours after PALA (Schedule II). We report here on the toxic and therapeutic results obtained. A preliminary report of Schedule I results was made in 1981 (31).

MATERIALS AND METHODS

Between September 1980 and February 1982, 32 patients were entered into Schedule I treatment, and, after an amendment, 19 patients were entered into Schedule II. The treatment protocol was approved by the Institutional Review Boards of Albert Einstein College of Medicine, Montefiore Hospital, and New York

[1] Received Mar 25, 1986; revised Oct 14, 1986; accepted Oct 28, 1986.
[2] This study was conducted by the Eastern Cooperative Oncology Group (*Paul P. Carbone*, chairman, CA-21115) and supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, and the Department of Health and Human Services.

[3] New York University Medical Center, New York (CA-16395).
[4] Albert Einstein College of Medicine, Bronx, NY (CA-14958).
[5] American Oncologic Hospital, Philadelphia, PA (CA-18281).
* *Reprint requests to:* Franco M. Muggia, MD, University of Southern California Cancer Center, 1441 Eastlake Ave, Los Angeles, CA 90033.

University's three hospitals. All patients had histologically documented unresectable, residual, or recurrent metastatic colorectal cancer with a performance status of ≥ 2 (ECOG scale) and measurable lesions. Eight patients, all in Schedule I, had received prior chemotherapy: five had had short courses of 5FU as an adjuvant, whereas three had had therapeutic courses of 5FU, 5FU plus semustine, and a combination of cyclophosphamide, methotrexate, and 5FU (CMF), respectively. None of the Schedule II patients had received prior chemotherapy. Only one patient in each schedule had received prior radiotherapy.

Treatment Schedule I consisted of 5FU, 400 mg/m² iv push/week, immediately preceded by a 30-minute infusion of PALA, 1.5 g/m² every other week. Dose escalations by 100 mg/m² were stipulated every 2 weeks to a maximum of 800 mg/m² if no or only grade 1 toxicity (32) was manifest. Beginning March 1981, a schedule change was introduced with PALA given weekly at a dose of 1.5 g/m² 1 day prior to 5FU. In this Schedule II, 5FU was begun at 300 mg/m², but escalated every two successive weekly doses to 400 mg/m² and subsequently to a maximum of 500 mg/m² if no toxicity was observed. Dose reduction (by 20%) was stipulated for 5FU after full recovery from grade 3 or 4 hematologic or gastrointestinal toxic effects [ECOG toxicity criteria (32)]. Therapeutic results were assessed utilizing standard criteria of response adopted by ECOG (32).

RESULTS

Patient characteristics were quite similar in the two schedules: the median ages in years were 60 and 63, respectively (range, 38–77), and a male-to-female ratio of 1. Patients had generally good performance status, and, with three exceptions in Schedule I, were not refractory to 5FU prior to this study. Only one patient on each schedule had received prior irradiation. In Schedule I, all but four patients had undergone surgical resection, whereas eight of 19 had not undergone surgery in Schedule II. Therapeutic effects according to schedule are shown in table 1 and indicate a major objective response rate of 18% (95% confidence intervals, 8%–30%). In Schedule II, efficacy evaluation was rendered difficult by violations (PALA given on same day as 5FU in four patients, and omitted every other week in three) or by failure to escalate dose of 5FU (14 escalations in Schedule I versus three in Schedule II). Both schedules were associated with early toxic withdrawals and prompted premature termination of Schedule II; < 25% of the patients remained on study after 6 months. Specific toxic events will be described.

Neurotoxicity.—This was severe in both schedules. Excluding weakness and fatigue, 15 (47%) patients in Schedule I and ten (53%) in Schedule II experienced neurotoxicity. Ataxia and lethargy were most frequent, but dysarthria, tremors, memory loss, and hallucinations were occasionally recorded. In two instances headaches were prominent, and one patient suffered from grand mal seizures. This last patient on Schedule II and four patients on Schedule I underwent extensive workup including computerized tomographic scans of the head and lumbar puncture for possible central nervous system involvement. One of these patients actually underwent placement of a ventriculojugular shunt because of diagnosis of hydrocephalus. No tumor block was ever found and the patient died of complicating bacterial (*Klebsiella*) meningitis. These patients were 62, 62, 48, 54, and 54 years old. The only feature in common was hepatomegaly and moderate liver involvement by tumor.

TABLE 1.—Therapeutic results*

	Schedule I	Schedule II
Entered	32	19
Prior therapeutic chemotherapy	3	0
Early toxic withdrawals†	6	5
Evaluable for response, not FU resistant	23	14
Complete remission	0	0
Partial remission (% evaluable)	6 (26)	3 (21)
Duration (mos)	9,9,6,4,3,2	9,6,5
Minor response	2	2
Duration (mos)	4,2	6,4
> 6 mos on study (%)	8 (25)	4 (22)
Overall major response (% total entered)	9 (18)	
(95% confidence intervals)	(8%–30%)	

*Unless otherwise indicated, values = No. of patients.
†Includes 1 toxic death (Schedule II) and 2 while tumor objectively shrinking (Schedule I).

Gastrointestinal (GI) toxicity.—These were the most common toxic effects, although at times difficult to separate from disease manifestations. Diarrhea, occasionally accompanied by abdominal pain and dehydration, occurred in 20 of 32 (62%) patients in Schedule I and 12 of 19 (63%) in Schedule II. Drug-related vomiting, mucositis, and intractable hiccups were recorded in 11 and seven, six and four, and two and no patients, respectively, on Schedules I and II.

Hematologic toxicity.—Hematologic changes were generally mild and became evident in Schedule I only after dose escalations of 5FU. On this schedule, wbc counts < 4000/mm³ occurred in nine patients, but none were < 2000/mm³. Platelet counts < 100,000/mm³ occurred in six. On Schedule II, only one patient experiencing sepsis followed by fatal obtundation and dehydration after the second dose escalation had hematologic toxicity with a wbc and platelet count of 1100 and 45,000/mm³, respectively. Only one patient on both schedules had a lower hematocrit, possibly in relation to chemotherapy, whereas several actually demonstrated increases in this parameter presumably from GI toxicity and dehydration.

Other toxic effects.—Skin, conjunctival, and nasal irritative phenomena were frequent. Most common was the appearance of an erythematous pruritic rash involving the nasolabial folds (eight and four patients in the two schedules, respectively). Hyperpigmentation was

common in these instances. Rhinitis, often leading to minor bleeding episodes, was reported by five and three. Conjunctivitis occurred in six and four, and alopecia occurred in two patients on each schedule. Five patients, all in Schedule I, had dysuria with pyuria and were treated for urinary tract infections. Two patients had vulvitis. Fever was prominent in six patients on Schedule I, and in two on Schedule II, but only in one instance was it complicated by neutropenia and probable sepsis (described under hematologic toxicity). One patient in each schedule suffered from phlebitis and a pulmonary embolus; hemoconcentration was present in both.

DISCUSSION

In view of likely biochemical interactions, exploitable effects between PALA and 5FU resulting in synergy were sought in cell lines and in animal tumors (17–23). Several phase I studies utilized a variety of schedules (24,25), and subsequently phase II studies (26–30) in colorectal cancer, breast cancer, and other tumors were performed. In addition, further modulation by concurrent use of thymidine has been carried out in a phase I study (33) and in a phase II study (34,35) in colorectal cancer. Review of these studies indicates remarkably similar GI, neurologic, and dermatologic events to those described, and some responses in colorectal (26–28,34), pancreatic (27), and breast cancer (27,30). However, in a large trial in colorectal cancer Buroker et al (35) found no differences in response rates or survival between 5FU alone and several combinations to modulate its effect including 5FU and PALA.

Prominent toxic effects from PALA plus 5FU led Martin and co-workers to explore additional selective modulation by employing uridine rescue of 5FU marrow and gut toxic effects (36) or methotrexate biochemical effects (37). Moreover, he and others have stressed the need for proper timing (PALA preceding 5FU by about 24 hours) and the ability to demonstrate relevant biochemical effects by low doses of PALA (38). They have stressed reproducing optimal preclinical conditions in study design but only later did they emphasize maximizing 5FU doses in any such attempts at modulations. The current study, in fact, differed from others in that it attempted to deliver maximal dosage of weekly 5FU through dose escalations every 2 weeks while a constant PALA dose below its presumed neurotoxic threshold from phase I experience was given biweekly (Schedule I). In spite of toxic effects observed on the initial schedule, antitumor effects led us to explore the 24-hour interval for PALA and 5FU postulated to be optimal (Schedule II). However, this second part was interrupted prematurely because of continued problems with toxic effects and patient compliance. Therefore, our conclusions do not differ from others, which is not surprising given that we were unable to exceed the 5FU dose rates of other studies.

The genesis of toxic manifestations from the combination is worth analyzing if one is to consider further manipulation of 5FU action by PALA and other agents. Pharmacokinetic studies in dogs indicate higher 5FU peak levels in the CSF in the presence of PALA (39). In these experiments, no other pharmacokinetic alterations were observed, but increased incorporation of FUTP in liver and intestine was also demonstrated. PALA by itself was neurotoxic in phase I trials but mostly when given in single dosing schedules at doses > 3 g. However, patients with preexisting central nervous system dysfunction had increased susceptibility to develop PALA-induced seizures and mental changes. Neurotoxicity from PALA is otherwise somewhat similar to that of 5FU, with ataxia and lethargy being prominent. GI toxicity occurs with either agent and is probably significantly worse when agents are combined. Mucositis may be severe with infusion schedules of PALA and would not be expected from dosages employed in this study. Additive or greater toxicity from both agents may account for the intractable diarrhea often accompanied by abdominal cramps. Similarly, conjunctivitis, rhinitis, and urethritis may be due to either agent, but most likely were rendered severe from the combined effect, since these are usually not described in 5FU trials. A rise in hematocrit with several of the patients also raises the question of hemoconcentration as a possible cause for thromboembolic complications noted in a previous study of PALA alone (15). Hematologic toxicity was not greater than would be expected from 5FU alone. As suggested by Martin and co-workers (38), considerably lower doses of PALA which still possess "modulating" activity need to be tested with therapeutic doses of 5FU. The dose schedule tested by Casper et al (24) could be utilized for future studies (40). In addition, greater understanding of the neurotoxicity of fluorinated pyrimidines might allow one to overcome these troubling effects.

In summary, in spite of discouraging results, one should not dismiss the possibility of useful synergy emerging for PALA plus 5FU. Experience in breast cancer, recently published (30), plus consistent responses observed in colorectal cancer in this and other studies raise hopes that further clinical investigation attempting to modulate 5FU might prove useful for patients with these metastatic cancers. Although results were not encouraging, in a five-arm randomized study including 5FU plus PALA and 5FU plus thymidine, toxicity patterns differed substantially from 5FU alone and each other to indicate the occurrence of modulation of effects on target tissues (35). Future research should explore the possibility that substantially lower doses of PALA might be sufficient to interact synergistically with 5FU in human tumors. Biochemical alterations such as inhibition of orotic aciduria induced by pyrazofurin have indeed been documented with doses of PALA < 250 mg/m^2 in man (24). Coupling this to other avenues of biochemical modulation of 5FU action might also be profitably explored with hopes that dose-limiting neurotoxic effects can be lessened while antitumor effects are retained or further enhanced.

REFERENCES

1. COLLINS KD, and STARK GR. Aspartate transcarbamylase. Interaction with the transition state analog *N*-(phosphonoacetyl)-L-aspartate. J Biol Chem 246:6599, 1977.
2. JOHNSON RK. Reversal of toxicity and antitumor activity of *N*-(phosphonoacetyl)-L-aspartate by uridine or carbamyl-DL-aspartate in vivo. Biochem Pharmacol 26:81–84, 1977.
3. ERLICHMAN CE, STRONG JM, WIERNIK PH, ET AL. Phase I trial of PALA (*N*-phosphonoacetyl-L-aspartate). Cancer Res 39:3992–3995, 1979.
4. HART RD, OHNUMA T, and HOLLAND JF. Initial clinical study with *N*-(phosphonoacetyl)-L-aspartic acid (PALA) in patients with advanced cancer. Cancer Treat Rep 64:617–624, 1980.
5. GRALLA RJ, CASPER ES, NATALE RB, ET AL. Phase I trial of PALA. Cancer Treat Rep 64:1301–1305, 1980.
6. ERVIN TJ, BLUM RH, MESHAD MW, ET AL. Phase I trial of *N*-(phosphonoacetyl)-L-aspartic acid (PALA). Cancer Treat Rep 64: 1067–1071, 1980.
7. VALDIVIESO M, MOORE EC, BURGESS AM, ET AL. Phase I clinical study of *N*-(phosphonoacetyl)-L-aspartic acid (PALA). Cancer Treat Rep 64:285–292, 1980.
8. KOVACH JS, SCHUTT AJ, MOERTEL CG, ET AL. Phase I study of *N*-(phosphonoacetyl)-L-aspartic acid (PALA). Cancer Treat Rep 63: 1909–1912, 1979.
9. RUBIN J, PURVIS J, BRITELL JC, ET AL. Phase II study of PALA in advanced large bowel carcinoma. Cancer Treat Rep 65:335–336, 1981.
10. EARHART RH, ELSON PJ, ROSENTHAL SN, ET AL. Phase II study of PALA and AMSA in advanced renal cell carcinoma. Am J Clin Oncol 6:555–560, 1983.
11. TAYLOR SG, IV, DAVIS RE, FALKSON GE, ET AL. PALA in advanced breast cancer: a phase II pilot study by ECOG. Am J Clin Oncol 5:627–629, 1982.
12. CREAGAN ET, EAGAN RT, FLEMING TR, ET AL. Phase II evaluation of PALA in patients with metastatic lung cancer. Cancer Treat Rep 65:356–357, 1981.
13. CASPER ES, GRALLA RJ, KELSEN DP, ET AL. Phase II evaluation of *N*-(phosphonoacetyl)-L-aspartic acid (PALA) in patients with non-small cell carcinoma of the lung. Cancer Treat Rep 64:705–707, 1980.
14. CREAGAN ET, NICHOLS WC, and O'FALLON JR. Phase II evaluation of PALA in patients with advanced head and neck cancer. Cancer Treat Rep 65:827–829, 1981.
15. MUGGIA FM, TSIATIS AA, O'CONNELL MJ, ET AL. Phase II trial oft PALA in lymphoma: an Eastern Cooperative Oncology Group study. Cancer Treat Rep 68:551–553, 1984.
16. CARROLL DS, GRALLA RJ, and KEMENY NE. Phase II evaluation of *N*-phosphonacetyl-L-aspartic acid (PALA) in patients with advanced colorectal carcinoma. Cancer Treat Rep 64:349–351, 1980.
17. MOYER JD, SMITH PA, LEVY EJ, ET AL. Kinetics of *N*-(phosphonacetyl)-L-aspartate and pyrazofurin depletion of pyrimidine ribonucleotide and deoxyribonucleotide pools and their relationship to nucleic acid synthesis in intact and permeabilized cells. Cancer Res 42:4525–4531, 1982.
18. LIANG CM, DONEHOWER RC, and CHABNER BA. Biochemical interactions between *N*-(phosphonacetyl)-L-aspartate and 5-fluorouracil. Mol Pharmacol 21:224–230, 1982.
19. SPIEGELMAN S, SAWYER R, NAYAK R, ET AL. Improving the antitumor activity of 5-fluorouracil by increasing its incorporation into RNA via metabolic modulation. Proc Natl Acad Sci USA 77:4966–4970, 1980.
20. KUFE DW, and EGAN EM. Enhancement of 5-fluorouracil incorporation into human lymphoblast ribonucleic acid. Biochem Pharmacol 30:129–133, 1981.
21. ARDALAN B, GLAZER RI, KENSLER TW, ET AL. Synergistic effect of 5-fluorouracil and *N*-(phosphonacetyl)-L-aspartate on cell growth and ribonucleic acid synthesis in a human mammary carcinoma. Biochem Pharmacol 30:2045–2049, 1981.
22. PETERS GJ, LAURENSSE E, LEYVA A, ET AL. The concentration of 5-phosphoribosyl 1-pyrophosphate in monolayer tumor cells and the effect of various pyrimidine antimetabolites. Int J Biochem 17:95–99, 1985.
23. JOHNSON RK, CLEMENT JJ, and HOWARD KS. Treatment of murine tumor with 5-fluorouracil in combination with de novo pyrimidine synthesis inhibitors PALA or pyrazofurin. Proc Am Assoc Cancer Res 21:292, 1980.
24. CASPER ES, VALE K, WILLIAMS LJ, ET AL. Phase I and clinical pharmacological evaluation of biochemical modulation of 5-fluorouracil with *N*-(phosphonacetyl)-L-aspartic acid. Cancer Res 43:2324–2329, 1983.
25. O'CONNELL MJ, POWIS G, RUBIN J, ET AL. Pilot study of PALA and 5-FU in patients with advanced cancer. Cancer Treat Rep 66:77–80, 1982.
26. BEDIKIAN AY, STROEHLEIN JR, KARLIN DA, ET AL. Chemotherapy for colorectal cancer with a combination of PALA and 5-FU. Cancer Treat Rep 65:747–753, 1981.
27. WEISS GR, ERVIN TJ, MESHAD MW, ET AL. Phase II trial of combination therapy with continuous-infusion PALA and bolus-injection 5-FU. Cancer Treat Rep 66:299–303, 1982.
28. ERLICHMAN C, DONEHOWER RC, SPEYER JL, ET AL. Phase I-phase II trial of *N*-phosphonacetyl-L-aspartic acid given by intravenous infusion and 5-fluorouracil given by bolus injection. JNCI 68:227–231, 1982.
29. PRESANT CA, ARDALAN B, MULTHAUF P, ET AL. Continuous 5-day infusion of PALA and 5FU: a pilot phase II trial. Med Pediatr Oncol 11:162–163, 1983.
30. MANN GB, HORTOBAGYI GN, BUZDAR AU, ET AL. A comparative study of PALA, PALA plus 5FU and 5FU in advanced breast cancer. Cancer 56:1320–1324, 1985.
31. CAMACHO FJ, MUGGIA FM, and KAPLAN BH. A trial of *N*-(phosphonacetyl)-L-aspartic (PALA) and 5-fluorouracil (5FU) in patients with metastatic colorectal cancer. Proc Am Assoc Cancer Res 22:457, 1981.
32. OKEN M, CREECH R, and TORMEY D. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649–655, 1982.
33. CHIUTEN DF, VALDIVIESO M, BEDIKIAN A, ET AL. Sequential administration of thymidine, 5-fluorouracil, and PALA—a phase I–II study. Am J Clin Oncol 8:332–335, 1985.
34. O'CONNELL MJ, MOERTEL CG, RUBIN J, ET AL. Clinical trial of sequential *N*-phosphonacetyl-L-aspartate, thymidine, and 5-fluorouracil in advanced colorectal carcinoma. J Clin Oncol 2:1133–1138, 1984.
35. BUROKER TR, MOERTEL CG, FLEMING TR, ET AL. A controlled evaluation of recent approaches to biochemical modulation or enhancement of 5-fluorouracil therapy in colorectal carcinoma. J Clin Oncol 3:1624–1631, 1985.
36. MARTIN DS, STOLFI RL, SAWYER RC, ET AL. High dose 5-fluorouracil with delayed uridine "rescue" in mice. Cancer Res 42:3964–3970, 1982.
37. MARTIN DS, STOLFI RL, SAWYER RC, ET AL. Improved therapeutic index with sequential *N*-phosphonacetyl-L-aspartate plus high-dose methotrexate plus high-dose 5-fluorouracil and appropriate rescue. Cancer Res 43:4653–4661, 1983.
38. MARTIN DS, STOLFI RL, SAWYER RC, ET AL. Therapeutic utility of utilizing low doses of *N*-(phosphonacetyl)-L-aspartic acid in combination with 5-fluorouracil: a murine study with clinical relevance. Cancer Res 43:2317–2321, 1983.
39. MILLER AA, MOORE C, HURLBERT RB, ET AL. Pharmacological and biochemical interactions of *N*-(phosphonacetyl)-L-aspartate and 5-fluorouracil in beagles. Cancer Res 43:2565–2570, 1983.
40. MARTIN DS. Biochemical modulation—perspective and objectives. *In* New Advances in Development of Cancer Chemotherapy: Bristol Myers Cancer Symposia, vol 8 (KR Harrap, ed). New York, Academic Press, 1986.

Local Neurotoxicity After Intra-arterial Cisplatin in Head and Neck Cancer[1]

Sergio Frustaci,* Luigi Barzan, Roberto Comoretto, Salvatore Tumolo, Giovanni Lo Re, and Silvio Monfardini[2]

Neurotoxicity after intra-arterial administration of cisplatin, alone or in combination with other chemotherapeutic agents, is not yet well-documented. In our experience with 63 previously untreated patients with head and neck cancer, we observed four patients with cranial nerve impairment ipsilateral to the cannulated artery. The first patient has already been reported, whereas the other three are the subject of the present report. Overall, all three patients developed cranial peripheral palsy a few days after the end of intra-arterial cisplatin and after a median total dose of 200 mg (range, 160–250). The nerves involved were the 12th in the first patient, the seventh in the second, and the ninth, tenth, 11th, and 12th in the third patient. Complete recovery of the palsy was noted only in the first patient. The patient reported previously had developed a cranial nerve palsy involving the ninth, tenth, 11th, and 12th nerves of the right side. The low incidence of this toxicity (6.3% in our experience) and the very high objective remission rate achieved by the intra-arterial administration of cisplatin justify the continuation of such an approach to obtain the maximum tumor regression and to research the individual predisposing factors. [Cancer Treat Rep 71:257–259, 1987]

Cisplatin was the first heavy metal compound showing antineoplastic activity in in vitro studies (1). Since its introduction in clinical use, cisplatin has shown significant activity in various malignancies such as testicular, ovarian, lung, and head and neck cancers (2).

On the other hand, similar to heavy metal poisoning, cisplatin has been associated with peripheral and central neurotoxic effects (3) such as paresthesias, loss of proprioception or vibratory sensation, retrobulbar neuritis, seizures, and damage of Corti's organ. Clinical ototoxicity is noted in up to half of the patients, appears to be dose- and age-related, and expresses as tinnitus and high-frequency hearing loss (4).

The exact mechanism by which cisplatin induces neurological toxicity is unclear, although segmental demyelination (2,5), loss of axons in the peripheral nerves (5), and denervation changes in the muscles (6) have been observed.

The intra-arterial administration of cisplatin in head and neck cancers, preliminary to definitive local treatment, began very recently and the clinical responses that were achieved are impressive (7–9). On the other hand, general and local toxic effects have been extensively reported by our group (9), whereas local neurological impairment has never been discussed.

We report here three patients with cranial nerve palsy that was observed during intra-arterial continuous infusion of low-dose cisplatin in head and neck cancers.

CASE REPORTS

Patient 1

Patient 1 was a 47-year-old woman who was accepted in our Otolaryngology Department after having several days of pain and otorrhea from the right ear. A seventh right cranial nerve palsy became evident 3 days later. Radiological evaluation, computerized tomographic and radionuclide scans, and exploratory surgery demonstrated a middle differentiated adenocarcinoma of the mastoid cell aditus. Intra-arterial chemotherapy and subsequent surgery were planned and the appropriate arterial cannulation was performed via the thyroid artery. A continuous infusion of cisplatin (10 mg every 12 hours for 10 consecutive days) was administered by an external infusion pump. Two days after the end of treatment a 12th cranial nerve palsy was observed. No other general or local toxic effects were noted. Twenty days after intra-arterial chemotherapy, radical surgery of the retromastoidal area and neck node dissection

[1] Received May 19, 1986; revised Oct 2, 1986; accepted Oct 28, 1986.

[2] Departments of Medical Oncology (*S. Frustaci, S. Tumolo, G. Lo Re,* and *S. Monfardini*) and Otolaryngology (*L. Barzan* and *R. Comoretto*), Centro di Riferimento Oncologico, Aviano (Pordenone), Italy.

* *Reprint requests to:* Sergio Frustaci, MD, Department of Medical Oncology, Centro di Riferimento Oncologico, Via Pedemontana Occ., 33081 Aviano (Pordenone), Italy.

were performed. The histology of the surgical specimens was negative for cancer; no node metastases were detected. The patient is alive and free of disease after 22 months, whereas the 12th cranial nerve palsy is immodified.

Patient 2

Patient 2 was a 70-year-old woman with an undifferentiated nasopharyngeal cancer involving the rhinopharynx and the skull base (Stage T4 N2 M0) who was treated with three cycles of systemic chemotherapy consisting of doxorubicin, vincristine, cyclophosphamide, and a nitrosourea. This combination determined a minor response, with disappearance of the palpable neck nodes. Before definitive radiation therapy we tried locoregional treatment. A right monolateral cannulation of the external carotid artery was performed because of the localization of the tumor mass. Cisplatin (10 mg every 12 hours for 8 days) in slow continuous infusion was administered. Treatment was then stopped because of mild renal impairment which subsequently completely recovered. On the tenth day a right peripheral seventh nerve palsy and alopecia of the right scalp were noted. A partial tumor mass regression was obtained. The patient was subsequently treated with radiation therapy (total dose, 7000 rads) and achieved complete clinical remission, pathologically confirmed at biopsy. Electromyogram of the right facial nerve revealed a total denervation of the inferior branch and a severe denervation of the superior branch. After 6 months the patient recovered the function of the superior branch with complete closing of the eyelid. However, after 12 months no recovery of the inferior branch has been noted.

Patient 3

Patient 3 was a 60-year-old man with a middle differentiated adenocarcinoma of the right ethmoid and maxillary sinus also involving the right orbita (Stage T4 N0 M0) who was treated with intra-arterial chemotherapy consisting of cisplatin (25 mg over 4 hours) and 5-fluorouracil (500 mg over 20 hours); both drugs were administered every day for 10 consecutive days. At the end of treatment, a mild mucositis of the oral cavity and pharynx, a limited area of skin necrosis at the neck level, and alopecia appeared together with a complete palsy of the ninth, tenth, 11th, and 12th right cranial nerves. No renal or hematological toxic effects were noted. After intra-arterial chemotherapy and radiation therapy (6000 rads), no clinical response was noted. After 9 months of follow-up the patient is still alive with persistent disease and only the 12th cranial nerve has recovered completely.

DISCUSSION

Intra-arterial chemotherapy for head and neck cancer began in the 1950s and the rationale for this treatment consists of increasing the drug concentration at the tumor level and, during prolonged infusion, possibly decreasing the systemic toxicity, together with inducing a continuous tumor cell exposure. Furthermore, intra-arterial chemotherapy can also induce an enhancement of the therapeutic index on the basis of the pharmacokinetic properties of the different drugs. In particular, the overall advantage of an intra-arterial administration of cisplatin can be evaluated as between two to five times greater in comparison with the iv administration (10). To our knowledge, there is a lack of data about neurological toxicity after intra-arterial administration of cisplatin. We have previously reported the first patient we observed (11). This patient, affected by a T3 N0 M0 of the right floor of the mouth, was treated by 20 mg of intra-arterial cisplatin for 10 days, obtaining complete clinical response of the tumor. However, 3 days after the end of treatment he developed a multiple ipsilateral cranial palsy involving the ninth, tenth, 11th, and 12th nerves and alopecia of the right temporal occipital region.

The three patients described here, together with the one previously reported, belong to our series of 63 patients treated from November 1980 with two consecutive protocols employing prolonged infusions of low-dose cisplatin. The first protocol, using cisplatin alone as a daily continuous infusion dose of 20 mg for 10–20 days, was used in 41 patients and their results have been recently reported (9). The second protocol, employing cisplatin (25 mg) as an infusion of 4 hours for 10 consecutive days in combination with other drugs, is now under investigation. All four patients reported are very similar. The neurotoxicity always involved one or more cranial nerves between the seventh and 12th, whereas the eighth was never affected. All investigations performed to establish the cause of the palsy proved negative and in particular computerized tomographic scan did not show any tumoral involvement of the nerves. Furthermore, the ipsilateral occurrence and the strict temporal relationship between intra-arterial cisplatin and neurological palsy are highly suggestive. In these four patients the median cisplatin dose administered was 200 mg (range, 160–250), whereas in the other 59 patients it was 250 mg (range, 160–400).

Neurological toxicity has been postulated as dose-dependent and therefore, during intra-arterial cisplatin, can be expected after high cumulative doses. In the four reported patients the cumulative dose was low and other mechanisms have to be considered. We can argue that the concentration per time ratio of the non-protein-bound cisplatin, the active and toxic species, could have also played a role. In fact, several papers have already reported the differences in cisplatin concentrations achieved by intra-arterial and iv administration (12–14). Moreover, individual factors could also have played a role in accounting for the low incidence of this toxicity and its appearance after a lower median cumulative dose in comparison with that of our entire population.

In conclusion, the very low incidence of this neurologi-

cal toxicity (6.3% in our total experience) justifies the continuation of our neoadjuvant intra-arterial treatment. However, prospective electromyographic studies are planned for all patients undergoing intra-arterial cisplatin to better define the individual or drug-related factors involved.

REFERENCES

1. ROSENBERG B, VAN CAMP L, and KRIGAS T. Inhibition of cell division in *Escherichia coli* by electrolysis products from a platinum electrode. Nature 205:698-699, 1965.
2. LOEHRER PJ, and EINHORN LH. Diagnosis and treatment. Drug five years later. Cisplatin. Ann Intern Med 100:704-713, 1984.
3. VON HOFF DD, SCHILSKY R, REICHERT CM, ET AL. Toxic effects of *cis*-dichlorodiammineplatinum(II) in man. Cancer Treat Rep 63: 1527-1531, 1979.
4. REDDEL RR, KEFFORD RF, GRANT JM, ET AL. Ototoxicity in patients receiving cisplatin: importance of dose and method of drug administration. Cancer Treat Rep 66:19-23, 1982.
5. BALOYANNIS SJ, and MANOLIDIS LS. Morphological alterations in cranial and spinal nerves by *cis*-platinum. Riv Orl Aud Fon 1:74-80, 1985.
6. HEMPHILL M, PESTRONK A, WALSH T, ET AL. Sensory neuropathy in *cis*-platinum chemotherapy. Neurology 30:429, 1980.
7. LEHANE DE, SESSIONS R, JOHNSON P, ET AL. (Abstr) Intra-arterial cisplatinum administration for advanced squamous cell carcinoma of the head and neck region. International Head and Neck Oncology Research Conference, Rosslyn, VA, 1980.
8. GALMARINI FC, YOCL J, ABULAFIA J, ET AL. Intra-arterial *cis*-platinum in head and neck cancer: controlled clinical trial in phase I and II. *In* Proceedings of the Annual Meeting of Europe Society Medical Oncology 6:18, 1980.
9. FRUSTACI S, BARZAN L, TUMOLO S, ET AL. Intra-arterial continuous infusion of *cis*-diamminedichloroplatinum in untreated head and neck cancer patients. Cancer 57:1118-1123, 1986.
10. COLLINS JM. Pharmacologic rationale for regional drug delivery. J Clin Oncol 2:498-504, 1984.
11. POMES A, FRUSTACI S, CATTAINO G, ET AL. Local neurotoxicity of cisplatin after intra-arterial chemotherapy: report of a case of unilateral multiple cranial nerve involvement. Acta Neurol Scand 73:302-303, 1986.
12. MAVLIGIT GM, BENJAMIN R, PATT YZ, ET AL. Intraarterial *cis*-platinum for patients with inoperable skeletal tumors. Cancer 48: 1-4, 1981.
13. STEWART DJ, BENJAMIN RS, ZIMMERMAN S, ET AL. Clinical pharmacology of intraarterial *cis*-diamminedichloroplatinum(II). Cancer Res 43:917-920, 1983.
14. GOUYETTE A, APCHIN A, FOKA M, ET AL. Pharmacokinetics of intra-arterial and intravenous cisplatin in head and neck cancer patients. Eur J Cancer Clin Oncol 22:257-263, 1986.

Thymidylate Synthase Gene Amplification in a Colon Tumor Resistant to Fluoropyrimidine Chemotherapy[1,2]

John L. Clark,[3,4] Sondra H. Berger,[5] Arnold Mittelman,[3] and Franklin G. Berger[5,6,*]

We have identified amplification of the thymidylate synthase gene in a colonic tumor that had developed resistance to 5-fluorouracil/leucovorin combination chemotherapy. The tumor had previously undergone a partial response to this combination but began to progress following a prolonged period of continuous therapy and relative disease stabilization. Since thymidylate synthase is a target enzyme for 5-fluorouracil, it is likely that the observed gene amplification is responsible for the resistance. Thus, gene amplification may be a relevant mechanism of acquired resistance to fluoropyrimidine chemotherapy in the clinic. [Cancer Treat Rep 71:261-265, 1987]

Resistance to chemotherapeutic drugs remains a major problem in the clinical management of neoplastic disease. Although a large body of information on the genetic, biochemical, and molecular nature of drug resistance has been gathered in studies of model cell lines and tumors, relatively little is known about resistance mechanisms operating in human tumors in vivo. Thus, there is need to expand existing information and to determine its applicability to the clinical setting.

Fluoropyrimidine antimetabolites, predominantly 5-fluorouracil (FUra) and 5-fluoro-2′-deoxyuridine (FdUrd), have been widely used in the treatment of carcinomas of the breast, ovary, and gastrointestinal tract (1). In general, these agents elicit an objective clinical response in only 20% of patients (2,3); the responses are usually transient and are followed by the development of resistance.

An important mechanism of fluoropyrimidine action involves formation of 5-fluoro-2′-deoxyuridylate (FdUMP), which is a potent inhibitor of thymidylate synthase (TS), an essential enzyme for DNA biosynthesis (4). TS catalyzes the reductive transfer of a methyl group from N^5N^{10}-methylenetetrahydrofolate (CH_2-THF) to dUMP to form thymidylate and dihydrofolic acid. The reaction occurs via a transient covalent complex among dUMP, CH_2-THF, and the enzyme (5). FdUMP, being an analog of dUMP, can also enter into complex formation; however, it cannot form product, so that the stable complex accumulates and inhibition of thymidylate formation results (4,5). The effectiveness of fluoropyrimidine inhibition of TS is determined, therefore, by a variety of parameters including, among others, FdUMP levels, CH_2-THF levels, and TS structure and concentration. This is borne out by a variety of studies with model cell lines and tumors (6–8). The significance of FdUMP formation and TS inhibition to clinical fluoropyrimidine therapy is still under debate.

In efforts to understand clinical resistance to fluoropyrimidines, we have been examining parameters related to TS inhibition in tumor samples from patients with colorectal carcinoma. In this report, we describe one patient whose tumor showed a fourfold to sixfold increase in TS gene copy-number after prolonged treatment with FUra in combination with leucovorin (CF). This result suggests that gene amplification, a well-recognized mechanism of drug resistance in model systems (8,9), may be important in the acquisition of resistance to fluoropyrimidine therapy in vivo.

MATERIALS AND METHODS

Clinical methods.—FUra/CF therapy (10) was performed as follows: CF was administered iv at a dose of 500 mg/m^2 over a 2-hour period; after the first hour of CF infusion, FUra (750 mg/m^2) was given by rapid iv injection. This treatment was performed weekly for 6 weeks, followed by a 2-week rest period.

Determination of TS level.—Tumor specimens were

[1] Received Aug 12, 1986; revised Oct 27, 1986; accepted Nov 4, 1986.

[2] Supported in part by Institutional Grant IN-54Y from the American Cancer Society. J. L. Clark was a recipient of a Clinical Oncology Career Development Award from the American Cancer Society.

[3] Department of Surgical Oncology, Roswell Park Memorial Institute, Buffalo, NY.

[4] *Present address:* Department of Surgery, University of Health Sciences, Chicago Medical School, North Chicago, IL.

[5] Departments of Basic Pharmaceutical Science (*S. H. Berger*) and Biology (*F. G. Berger*), University of South Carolina, Columbia.

[6] We thank Dr. Lewis Bowman for help with densitometric scanning and Teresa Parton-Lopes for secretarial work.

* *Reprint requests to:* Franklin G. Berger, Department of Biology, University of South Carolina, Columbia, SC 29208.

delivered to the laboratory on ice within 30 minutes of surgery and were either processed immediately or frozen at −70°C until further use. To prepare extracts for enzyme assay, tumors were thawed and ground up in a Dounce homogenizer in 2.5 volumes (wt/vol) of 0.2 M Tris (pH 7.4) containing 20 mM β-mercaptoethanol. The mixture was further homogenized by sonication (8) and subjected to centrifugation at 105,000 × *g* for 1 hour. The protein concentration in the supernatant was determined by the method of Lowry et al (11). TS levels were assayed by binding of [6-^{3}H]-FdUMP (20 Ci/mmol; Moravek Biochemicals, City of Industry, CA), as described previously (8). To minimize hydrolysis of FdUMP, the reaction mixtures included 17 mM CMP and 28 mM NaF (12).

Extraction and analysis of tumor DNA.—Total DNA was isolated from tumor samples by the method of Pellicer et al (13). For Southern blotting (14), DNAs (10 μg/sample) were digested with restriction nuclease *EcoRI*, fractionated by agarose gel electrophoresis, transferred to nitrocellulose, and hybridized to ^{32}P-labeled pMTS-3 DNA, which contains a cDNA insert complementary to mouse TS mRNA (15). Hybridizations were carried out at standard stringency, which was 0.6 M NaCl at 65°C. Blots were washed in 0.3 M NaCl at 65°C, and TS-specific DNA fragments were observed by autoradiography. As an internal control, 20 pg of pBR322 DNA was added to the digestion mixtures. Quantitation of relative labeling of the TS structural gene fragments on the autoradiograms was done using a Quick Scan R and D densitometer (Helena Laboratories, Beaumont, TX); peak integration was by computer, using Chromatochart Software (Interactive Microwave, Inc, State College, PA).

RESULTS

Patient History

Patient M, a 63-year-old male, underwent a sigmoid resection in 1977 to treat a colonic adenocarcinoma. A recurrent tumor was discovered in September 1980, at which time a transverse colostomy was performed. In November 1980, this patient was placed on FUra and radiation therapy in attempts to control an abdominal mass of the lower left quadrant. No response was observed and the mass was resected in December 1980. In September 1981, he was admitted to Roswell Park Memorial Institute with a metachronous primary tumor in the right colon, for which he underwent a right hemicolectomy. Subsequently, abdominal wall and left pelvic local recurrence of the original adenocarcinoma developed. Standard chemotherapeutic protocols, including FUra, were undertaken between November 1981 and December 1982 in attempts to control progression of the two tumors. No response to any of the therapies was observed, and the patient's overall health continued to deteriorate.

In February 1983, a FUra/CF combination protocol was begun. This combination has elicited enhanced response rates (40%–50%) in several clinical trials (10,16). The increased effectiveness of FUra/CF over FUra alone is probably due to expansion of the reduced folate pool by CF, which maximizes the binding of FdUMP into the ternary complex (5,17). The tumors in Patient M underwent a regression in surface area during the first 11 months of FUra/CF therapy; this was maintained with slight, if any, progression over the next 16 months of continued treatment. Performance status and overall health improved considerably and remained good throughout the therapeutic period. By July 1985, the tumors began to progress at an accelerated rate, and chemotherapy was discontinued in September. One of the masses was biopsied and a small sample was delivered to our laboratory. In all, Patient M had received > 100 courses of FUra/CF therapy during a 2½-year period. He died in June 1986. All other tumors in this study were from patients that had received no prior chemotherapy.

The tumor samples were divided in half and stored at −70°C until use. One piece was used for enzyme studies, while the other was used for DNA studies, as described below.

TS Enzyme Levels in Colorectal Tumors

We determined the concentration of TS enzyme in extracts prepared from tumor M, as well as from tumors from other patients. TS levels were measured by the ^{3}H-FdUMP binding assay; since FdUMP enters into a specific and stable covalent ternary complex with TS in the presence of CH_2-THF (5), the extent of FdUMP binding is a specific measure of TS concentrations in the extracts. To minimize problems associated with the presence of FdUMP phosphatase, we included NaF and CMP in the assays. The former is a general phosphatase inhibitor, while the latter is a competitive substrate for FdUMP phosphatase (12); thus, these agents maintain high FdUMP levels in the assays and maximize binding.

TS levels in a number of colon tumor extracts are

TABLE 1.—TS enzyme levels in colorectal tumors*

Tumor	TS concentration, pmols/mg of soluble protein
A	0.17 (0.11)
B	0.16 (0.04)
C	0.26 (0.03)
D	0.35 (0.05)
E	0.50
F	0.45 (0.04)
G	0.38 (0.06)
H	0.13 (0.04)
I	0.26 (0.05)
J	0.70 (0.03)
K	0.13 (0.02)
L	0.38
M	0.18 (0.03)

*TS was quantitated in tumor extracts by the ^{3}H-FdUMP binding method (*see* the Materials and Methods section for details). Nos. in parentheses = values obtained when NaF and CMP were not included in the assay.

shown in table 1. Values ranged from 0.13 to 0.70 pmols of TS/mg of protein, with the mean ± SD being 0.31 ± 0.17. The extracts that were assayed in the absence of NaF and CMP exhibited wide variation in the extent of reduction of FdUMP binding (table 1), consistent with the notion that FdUMP phosphatase levels vary greatly among colon tumors (18). The TS level in tumor M was not markedly different from the others.

Electrophoretic analysis of the TS complex in several of the tumors indicated that all of the radioactivity migrated as a single protein species having a molecular weight of 37,000 (data not shown). This is the known size for human TS (8), indicating that the FdUMP binding assay is a reliable method for detecting TS in the colon tumor extracts.

Amplification of the TS Structural Gene in Tumor M

We performed Southern blot analysis (14) to examine the structure and copy-number of the TS gene in DNA from colorectal tumors. Tumor DNAs were digested with restriction nuclease *EcoRI*, and the resulting fragments were fractionated by agarose gel electrophoresis and transferred onto a nitrocellulose membrane. The blots were incubated with a ^{32}P-labeled nucleic acid probe specific for TS sequences. The probe, pMTS-3, is a pBR322-derived plasmid containing a cDNA insert that corresponds to mouse TS mRNA (15); pMTS-3 cross-hybridizes with human TS sequences under normal stringency conditions (8).

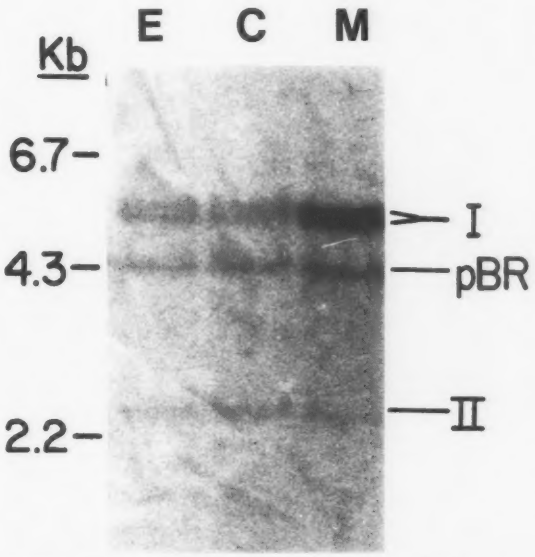


FIGURE 1.—Analysis of TS gene copy-number. Tumor DNAs from several patients were digested with restriction endonuclease *EcoRI* and analyzed by Southern blotting [(14); *see* Materials and Methods section]. The various DNA fragments are marked as follows: I, TS gene doublet; II, TS pseudogene (*see* text); pBR, plasmid pBR322 DNA, added as an internal control. Molecular weight standards, in kilobases (Kb), are indicated to the left.

TABLE 2.—Quantitation of TS gene copy-number*

Tumor	Areas (arbitrary units)		Normalized ratio
	Peak I	Peak II	
E	1086	484	1.0
C	925	633	0.65
M	5275	591	4.0

*Lanes E, C, and M from the Southern blot in fig 1 were scanned, and peak areas were integrated by computer (*see* Materials and Methods section for details). Peak I is the TS gene doublet at 5.5 kb, while peak II is the TS pseudogene fragment at 2.3 kb. The ratios were normalized to that of E, which was set at 1.0.

Figure 1 shows an autoradiogram of three tumor DNA digests. The doublet at 5.5 kb, marked I in figure 1, represents two fragments of the TS gene (8). The 2.3-kb fragment, marked II, is probably a TS pseudogene; it is not linked to the normal TS gene (R. Nussbaum, personal communication) and does not undergo amplification in FdUrd-resistant human cells that overproduce TS (8). This fragment, therefore, provides an internal control for any variation in DNA concentrations from lane to lane. As a further control, we added a small amount of pBR322 DNA to each digestion. The corresponding fragment, marked pBR, migrates as a 4.3-kb species. Qualitatively, the fragment patterns of the three tumors were virtually identical, indicating that the TS gene in tumor M does not contain major sequence alterations. However, it is clear that the hybridization of pMTS-3 to the doublet was significantly greater in DNA from tumor M than in the other tumors, while hybridization to fragment II or to pBR322 was essentially unchanged in all samples. This indicates a higher TS gene copy-number in tumor M. Quantitation of band intensities by densitometric scanning of the autoradiograms (table 2) indicated a fourfold to sixfold amplification of the TS gene in tumor M relative to tumors E and C. These results suggest that the fluoropyrimidine resistance exhibited by tumor M may have been generated by a gene amplification mechanism.

DISCUSSION

We have identified amplification of the TS gene in DNA from a fluoropyrimidine-resistant tumor that had previously responded to therapy. After > 100 courses of FUra/CF treatment, which elicited partial tumor regression and relative disease stabilization over a 2½-year period, the tumor became resistant to further fluoropyrimidine chemotherapy and exhibited a fourfold amplification of the TS gene. We cannot conclude definitively that the amplification occurred during treatment, since no tumor samples were available before FUra/CF therapy. However, in light of the observed low TS gene copy-number in DNA from nine other tumors (fig 1 and data not shown), it is unlikely that the

amplification existed prior to therapy. Further studies will have to address this issue in more detail.

It is interesting to note that the TS enzyme level in tumor M was similar to that in 12 other tumors (table 1). Thus, the fourfold amplification was not associated with a high level of enzyme. Any of a number of models might be proposed to explain this finding. One possibility is that prior to therapy, the drug-responsive tumor cells had a relatively low TS concentration. Following gene amplification, an induction of TS to a level similar to that in the other tumors may have occurred, resulting in resistance to further treatment with the FUra/CF combination. The TS concentration, therefore, may be an important determinant of clinical response to fluoropyrimidine chemotherapy, a notion consistent with conclusions drawn from studies of model tumor cell lines of gastrointestinal origin [(19); J. Clark, S. Berger, and F. Berger, manuscript in preparation].

The results reported here do not address the role of possible cellular heterogeneity within colorectal tumors. In addition, we have not determined the ratio of neoplastic to normal cells in our samples. Thus, the observed gene amplification may be an average value. Some cells within the tumor may have greater than fourfold amplification, while others may have none. The fact that the tumor underwent partial regression indicates that only a fraction of the cells were removed by drug treatment; the remainder must have been either growth-arrested tumor cells or nonneoplastic stromal cells. The observed progression during relapse may have been due, therefore, to a subpopulation of cells within the tumor.

Specific gene amplification is a very common mechanism of drug resistance and has been observed in cultured cell lines selected for resistance to a wide variety of cytotoxic drugs, including fluoropyrimidines, in vitro (8,9). The process results in overproduction of critical gene products that allow the cell to overcome the effects of the drug. Our present findings, along with reports of amplification of the dihydrofolate reductase gene in leukemic cells from patients undergoing methotrexate therapy (20,21), indicate that gene amplification may be a general mechanism of clinical resistance to chemotherapy.

Fluoropyrimidine action can involve mechanisms other than FdUMP formation and TS inhibition. The synthesis of FUTP followed by its incorporation into RNA has been considered an equally important mode of FUra toxicity (22). The relative significance of TS inhibition versus RNA incorporation in clinical therapy has been debated extensively. Combinations of FUra with agents that are directed at increasing FUra incorporation into RNA have not been of therapeutic benefit (3,23). In contrast, the combination of FUra with CF, designed to maximize FdUMP binding into an inhibitory complex with TS, has shown enhanced therapeutic efficacy in several independent clinical trials (10,16). The success of this combination most likely derives from an increase in the pool of reduced folates, which is critical to the tight binding of FdUMP and TS (5,17). The present finding of TS gene amplification in a patient receiving prolonged FUra/CF therapy suggests that the target of this combination is, indeed, TS.

Further studies will be directed at determining the general significance of TS gene amplification, as well as other parameters related to FdUMP inhibition of TS, in clinical response to fluoropyrimidine chemotherapy. In so doing, it is expected that the clinical relevance of drug response parameters identified in model studies can be assessed and that potential biochemical and/or molecular markers for response can be evaluated.

REFERENCES

1. HEIDELBERGER C. Fluorinated pyrimidines and their nucleosides. *In* Antineoplastic and Immunosuppressive Agents. Handbook of Experimental Pharmacology (Sartorelli C, and Johns DG, eds). New York, Springer-Verlag, 1974, vol 38, pp 193–231.
2. MOERTEL CG. Current concepts in cancer: chemotherapy of gastrointestinal cancer. N Engl J Med 299:1049–1052, 1978.
3. PETRELLI NJ, and MITTELMAN A. An analysis of chemotherapy for colorectal carcinoma. J Surg Oncol 25:201–206, 1984.
4. DANENBERG PV. Thymidylate synthetase—a target enzyme in cancer chemotherapy. Biochim Biophys Acta 473:73–92, 1977.
5. SANTI DV, MCHENRY CS, and SOMMER M. Mechanism of interaction of thymidylate synthetase with 5-fluorodeoxyuridylate. Biochemistry 13:471–480, 1974.
6. HOUGHTON JA, MARODA SJ, PHILLIPS JO, ET AL. Biochemical determinants of responsiveness to 5-fluorouracil and its derivatives in xenografts of human colorectal adenocarcinomas in mice. Cancer Res 41:144–149, 1981.
7. BAPAT AR, ZAROW C, and DANENBERG PV. Human leukemic cells resistant to 5-fluoro-2′-deoxyuridine contain a thymidylate synthetase with lower affinity for nucleotides. J Biol Chem 258:4130–4136, 1983.
8. BERGER SH, JENH C-H, JOHNSON LF, ET AL. Thymidylate synthase overproduction and gene amplification in fluorodeoxyuridine-resistant human cells. Mol Pharmacol 28:461–467, 1985.
9. SCHIMKE RT. Gene amplification, drug resistance, and cancer. Cancer Res 44:1735–1742, 1984.
10. MADAJEWICZ S, PETRELLI N, RUSTUM YM, ET AL. Phase I–II trial of high-dose calcium leucovorin and 5-fluorouracil in advanced colorectal cancer. Cancer Res 44:4667–4669, 1984.
11. LOWRY OH, ROSEBROUGH NJ, FARR AL, ET AL. Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275, 1951.
12. SPEARS CP, SHAHINIAN AH, MORAN RG, ET AL. In vivo kinetics of thymidylate synthetase inhibition in 5-fluorouracil-sensitive and -resistant murine colon adenocarcinomas. Cancer Res 42:450–456, 1982.
13. PELLICER AM, WIGLER M, AXEL R, ET AL. The transfer and stable integration of the HSV thymidine kinase gene into mouse cells. Cell 14:133–141, 1978.
14. SOUTHERN EM. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98:503–517, 1975.
15. GEYER PK, and JOHNSON LF. Molecular cloning of DNA sequences complementary to mouse thymidylate synthase messenger RNA. J Biol Chem 259:7206–7211, 1984.
16. MACHOVER D, SCHWARZENBERG L, GOLDSCHMIDT E, ET AL. Treatment of advanced colorectal and gastric adenocarcinomas with 5-fluorouracil combined with high-dose folinic acid: a pilot study. *In* Current Drugs and Methods of Cancer Treatment (Mathé G, Mihich E, and Reizenstein P, eds). New York, Masson Publishing, 1983, pp 111–114.
17. LOCKSHIN A, and DANENBERG PV. Biochemical factors affecting the tightness of 5-fluorodeoxyuridylate binding to human thymidylate synthetase. Biochem Pharmacol 30:247–257, 1981.
18. WOLBERG WH, and MORIN J. Thymidylate synthetase activity and

fluorouracil sensitivity of human colonic cancer and normal mucosal tissue preparations. Cancer 47:1313–1317, 1981.
19. WASHTIEN W. Thymidylate synthetase levels as a factor in 5-fluorodeoxyuridine and methotrexate cytotoxicity in gastrointestinal tumor cells. Mol Pharmacol 21:723–728, 1982.
20. HORNS RC, DOWER WJ, and SCHIMKE RT. Gene amplification in a leukemic patient treated with methotrexate. J Clin Oncol 2:2–7, 1984.
21. CARMAN MO, SCHORNAGEL JH, RIVEST RS, ET AL. Resistance to methotrexate due to gene amplification in a patient with acute leukemia. J Clin Oncol 2:16–20, 1984.
22. WILKINSON DS, and CRUMLEY J. Metabolism of 5-fluorouracil in sensitive and resistant Novikoff hepatoma cells. J Biol Chem 252: 1051–1056, 1977.
23. AU JLS, RUSTUM YM, LEDESMA EJ, ET AL. Clinical pharmacologic studies of continuous infusion of 5-fluorouracil and thymidine preceded by a bolus dose of thymidine in treatment of colorectal carcinoma. Cancer Res 42:2930–2937, 1982.

Experimental Dacarbazine Antitumor Activity and Skin Toxicity in Relation to Light Exposure and Pharmacologic Antidotes[1,2]

Robert T. Dorr,* David S. Alberts, Janine Einspahr, Nancy Mason-Liddil, and Michelle Soble[3]

Dacarbazine (DTIC) is reported to exhibit enhanced clinical toxicity and increased antitumor activity in vitro when exposed to light. Since it was unclear whether light exposure enhanced DTIC antitumor activity or local toxic effects in vivo, a series of experiments was performed in mice given DTIC solutions exposed to light for 2 hours at room temperature. Adenocarcinoma 07/A was implanted by trocar in adult female BALB/c mice. DTIC (50 and 100 mg/kg) was given ip three times per week for 2 weeks. Both drug doses significantly inhibited tumor growth. However, there was no significant difference between light-exposed and -protected drug treatments. In vitro clonogenic assays in L1210 leukemia and Chinese hamster ovary (CHO) cells demonstrated that DTIC cytotoxicity was not increased with light exposure (0.8 J/m^2/sec). Both cell lines showed a dose-response relationship to DTIC after 1- or 6-hour exposures in the presence or absence of light. Normal dehaired BALB/c mice were given single intradermal injections of 0.5, 1.75, 5.0, or 10 mg of DTIC in 0.05 ml of saline. Dose-dependent skin ulceration was produced at the 1.75-, 5.0-, and 10.0-mg dose levels. Again, there was no consistent statistical difference in skin ulceration between treatments using light-exposed and -protected DTIC vials. However, when *mice* were exposed to light following intradermal DTIC, increased skin toxicity was produced ($P < 0.05$ by Student-Neuman-Keuls multiple range test). A number of potential local antidotes to DTIC skin ulceration were found to be ineffective. These included: L-cysteine, dimethyl sulfoxide, hyaluronidase, hydrocortisone, and 0.9% saline. Sodium thiosulfate (0.3 M) significantly reduced DTIC skin ulcers as did pre-exposure of DTIC to S-9 rat liver enzymes and NADPH. Neither mild skin heating nor cooling reduced DTIC ulcerations. DTIC appears to synergize with light in vivo to produce increased toxicity. Patients receiving DTIC should avoid intense light exposure after drug injection. However, elaborate precautions to prevent light exposure of DTIC solutions during preparation or injection appear to be unnecessary.
[Cancer Treat Rep 71:267-272, 1987]

Dacarbazine (DTIC), a purine analog, is an anticancer agent (1). In vivo, DTIC does not act as an antimetabolite and it is relatively inactive in isolated tissue culture systems (1,2). However, following metabolic activation by microsomal NADPH-dependent mixed-function oxygenases, several methylating intermediates are formed, and the main drug action is believed to involve alkylation of DNA (3). In the presence of light, a DTIC photodecomposition product, 5-diazoimidazole-4-carboxamide, may cyclize to the purine analog 2-azahypoxanthine (1). Both 5-diazoimidazole-4-carboxamide and 2-azahypoxanthine are directly cytotoxic in tissue culture (4). Thus, photoactivation of DTIC has been used to explain some of the uncommon clinical toxic effects of the drug including local venous pain, a flu-like syndrome, nausea and vomiting (4), and rare photosensitivity reactions (5). Because it was unclear whether light exposure of DTIC solutions played a role in the drug's antitumor efficacy or toxicity in vivo, the following DTIC antitumor and skin toxicity experiments were conducted to answer this question.

MATERIALS AND METHODS

BALB/c adult female mice (Jackson Laboratories, Bar Harbor, ME) were used in these experiments. DTIC (Dome) was obtained in commercial 100-mg vials of lyophilized drug from Dome Division, Miles Laboratories

[1] Received Jan 30, 1985; revised Oct 28, 1986; accepted Nov 20, 1986.
[2] Supported in part by Public Health Service grants CA-31078, CA-23074, and CA-14612 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] Department of Medicine, Arizona Cancer Center, College of Medicine, The University of Arizona, Tucson.
* *Reprint requests to:* Robert Dorr, PhD, Arizona Cancer Center, The University of Arizona, 1515 N Campbell Ave, Tucson, AZ 85724.

Inc, West Haven, CT. For in vivo antitumor studies, 100-mg vials were initially diluted with 9.9 ml of sterile water for injection, USP, as recommended by the manufacturer to yield a concentration of 10 mg/ml. This solution was used for antitumor studies at a dose of 100 mg/kg. For 50-mg/kg treatments, this initial solution was diluted 1:1 in sodium chloride injection (0.89%), USP. All drugs were prepared freshly in 0.89% sodium chloride each day, immediately prior to use. Ip injections were given at 0.1 ml/10 g and intradermal injections of 200, 100, 35, and 10 mg/ml were given in a constant 0.05-ml volume. The intradermal DTIC injections were given once into a hair-free site (1 × 1 cm) on the dorsum of the mouse (6).

DTIC solutions for light pre-exposure studies were transferred to clear glass vials (Hollister-Stier, Spokane, WA) and left to stand for 2 hours at room temperature at a distance of 30 cm from four opposing 150-W Sylvania photography bulbs, each positioned at 90° angles to the vials. This elevated solution temperature by approximately 5°C. Chemical loss of DTIC was not measured. Light-protected vials were kept in the original amber glass containers at room temperature and were used within 2 hours of reconstitution. For in vivo post-exposure light studies, BALB/c mice were injected intradermally with DTIC and placed into light-protected or -exposed cages, each for 24 hours. Light exposure (total integrated spectrum) was quantitated at approximately 0.05 J/m^2/sec. Potential local DTIC antidotes were given intradermally, proximal to the DTIC intradermal injection site. These included L-cysteine (Sigma Chemical, St. Louis, MO); dimethyl sulfoxide (DMSO) (Malinckrodt); hyaluronidase (Wydase, Wyeth Labs, Pittsburgh, PA); hydrocortisone (Solu-Cortef, The Upjohn Co, Kalamazoo, MI); and sodium thiosulfate, USP (Torigian Labs, Queens Village, NY). Topical heat or cold application was also tested as a potential DTIC antidote. This involved either a 30-minute hot (43°C–44°C) or cold (9°C–10°C) exposure to the intradermal DTIC site using special Plexiglas mouse restraints reported in detail elsewhere (7).

Microsomal enzyme activation of DTIC followed the procedure of Ames et al (8) using 100 mg of drug with 40 mg of a commercial S-9 rat liver homogenate (Litton Bionetics, Charleston, SC) with an NADPH-generating system [containing per milliliter: 5 mg of NADP, 15 mg of glucose 6-PO_4, and 50 L each of $MgCl_2$ (0.1 M) and glucose-6-phosphate dehydrogenase (100 U of activity)]. The incubation mixture was maintained at 37°C and ambient oxygen tension for 30 minutes. The microsomal enzymes were then removed by centrifugation (1000 × g for 10 mins).

For the in vivo antitumor studies, the 07/A colon cancer line was obtained from Dr. Robert Struck of the Southern Research Institute, Birmingham, AL. The tumor fragments (30–35 mg) were implanted by trocar sc into the right flank of the mice. Two days after implantation, a series of six ip DTIC injections were administered on a three times per week schedule.

The interaction of light with DTIC was also evaluated in vitro using a two-layer soft agar cloning technique (9). Two cell lines, L1210 leukemia and Chinese hamster ovary (CHO) cells, were exposed to DTIC in vitro for 1 or 6 hours in the presence or absence of intense white light (the same as for drug activation). Light exposure was quantitated using a shortwave Blackray ultraviolet meter (Model J-225, Ultraviolet Products Inc, San Gabriel, CA). During the light-exposure experiments, cells in medium with serum (4 × 10^4/ml L1210 and × 10 CHO) were placed in uncovered 35-mm Petri dishes under class 100 laminar airflow conditions. DTIC concentrations were 10–1000 mg/ml diluted into pH 7.4 phosphate-buffered saline. Following the 1- or 6-hour exposure period, cells were washed free of drug by centrifugation twice at 800 × g for 10 minutes and plated in 0.3% agar in triplicate into 35-mm Petri dishes. The plates were incubated at 37°C in 10% CO_2/90% air for 5–10 days. CHO cells were grown in McCoy's 5A medium containing 10% fetal bovine serum. L1210 cells were similarly grown in RPMI-1640 medium (GIBCO). Colonies ≥ 60 μm in size were counted by an automated image analysis system (Omnicon, FAS-II, Bausch and Lomb, NY). Survival of drug-treated tumor colony-forming units was compared to untreated controls which were also exposed to light for 1 or 6 hours prior to plating. Each experiment was performed at least twice.

For the initial skin toxicity studies, six groups of five normal mice each (light-exposed and -protected) were evaluated at three intradermal DTIC doses: 0.5, 1.75, 5, and 10 mg (corresponding to DTIC doses of approximately 71, 250, 710, and 1420 mg/m^2) (10). DTIC and potential local antidotes were given in 0.05 ml of 0.9% sodium chloride. These agents included L-cysteine (10 mM), topical DMSO (100%), hyaluronidase (10 U), hydrocortisone (2.5 mg), sodium thiosulfate (0.17–0.33 M), and 0.05 ml of the S-9/NADPH mixture. Topical heating to 43°C–44°C or cooling to 8°C–10°C each for 30 minutes was also evaluated for local antidotal effects in unanesthetized animals. Skin lesions were evaluated daily by a single observer for the widest perpendicular widths of induration, erythema, and ulceration.

Survival statistics for the in vivo antitumor studies were calculated by both the Wilcoxon method, which censors data to accentuate early survival differences (11), and the log-rank method, which emphasizes late survival differences (12). The statistical tests for the skin toxicity studies included an initial analysis of variance with a subsequent intergroup multiple range test comparison using the Student-Neuman-Keuls (SNK) procedure. Significance for all statistical analyses was $P \leq 0.05$.

RESULTS

In Vivo Antitumor Studies

Tumor implantation was successful in all animals but two in the 50-mg/kg group and in one animal in the 100-mg/kg group. Table 1 shows the survival data for

TABLE 1.—Survival of mice bearing adenocarcinoma 07/A treated with DTIC

Group No.	Treatment	DTIC light exposure*	No. of mice	60-day survivors (%)	Statistical comparison of groups	*P* value: Log-rank	*P* value: Wilcoxon
1	Control		10	10			
2	All DTIC treatments	+, −	40	40	1 vs 2	0.001	0.002
3	All, 50 mg/kg	+, −	20	30	1 vs 3	0.008	0.006
4	All, 100 mg/kg	+, −	20	50	1 vs 4	0.002	0.003
					3 vs 4	0.221	0.245
5	All DTIC, 50 mg/kg	−	20	25			
6	All DTIC treatments	+	20	55	5 vs 6	0.076	0.118
7	DTIC, 50 mg/kg	−	10	20	7 vs 1	0.057	0.031
8	DTIC, 50 mg/kg	+	10	40	7 vs 8	0.487	0.706
					8 vs 1	0.017	0.014
9	DTIC, 100 mg/kg	−	10	30	9 vs 1	0.057	0.054
10	DTIC, 100 mg/kg	+	10	70	9 vs 10	0.078	0.083

*+ = light-exposed; − = light-protected.

the antitumor studies. Two months after tumor implantation, 10% of control mice were alive as compared to 30% of mice given 50 mg/kg ($P = 0.005$) and 50% of mice given 100 mg/kg ($P < 0.005$). Collectively, the DTIC treatments (ie, both 50 and 100 mg/kg, light-protected and -exposed) were significantly different from the control group by either the Wilcoxon or log-rank method. However, there were no statistically significant survival differences between the two different DTIC treatment groups. When directly compared, light-exposed and -protected treatment groups exhibited no statistically significant survival differences. Thus, light-protected DTIC solutions produced fewer long-term survivors, but this trend did not reach a statistically significant magnitude.

In Vitro Antitumor Studies

Figure 1 shows the results of the in vitro colony-forming assays of DTIC activity. L1210 cells exposed to DTIC for 1 or 6 hours (fig 1A) did not demonstrate light-enhanced cytotoxicity. Significant cell killing (≥ 50% decrease in colony formation) was noted at 1-hour DTIC concentrations of 500 μg/ml. Similar results were produced with the 6-hour exposure. CHO cells appeared to be slightly less sensitive to DTIC, with no significant cell killing at doses < 750 μg/ml of DTIC for 1 or 6 hours (fig 1B). Again, there was no evidence for light-enhanced cell killing, using either a pre-exposure of DTIC *vials* to light or a concurrent DTIC-light exposure of cells. Light exposure reference calibrations demon-

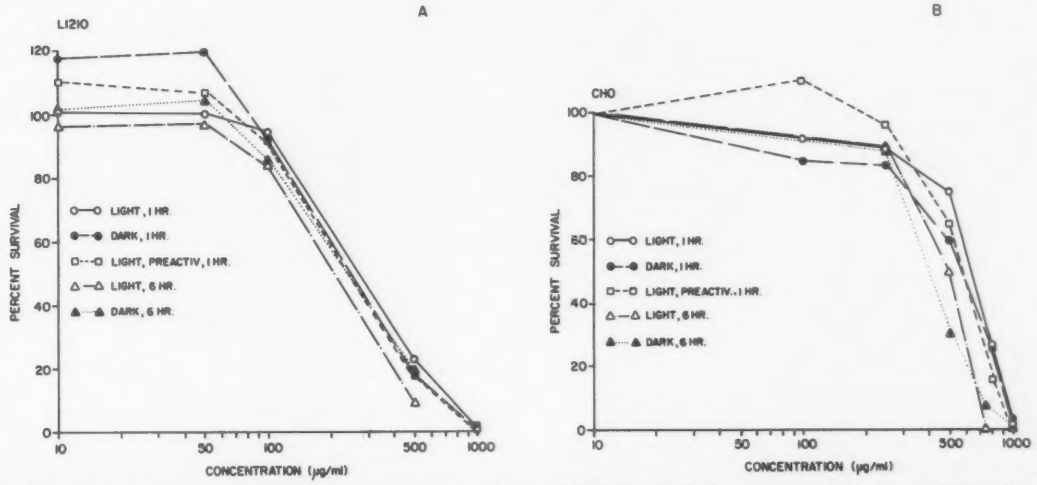


FIGURE 1.—Survival of tumor colony-forming units for L1210 leukemia (A) and CHO (B) cells. Each point represents the mean of 3 determinations.

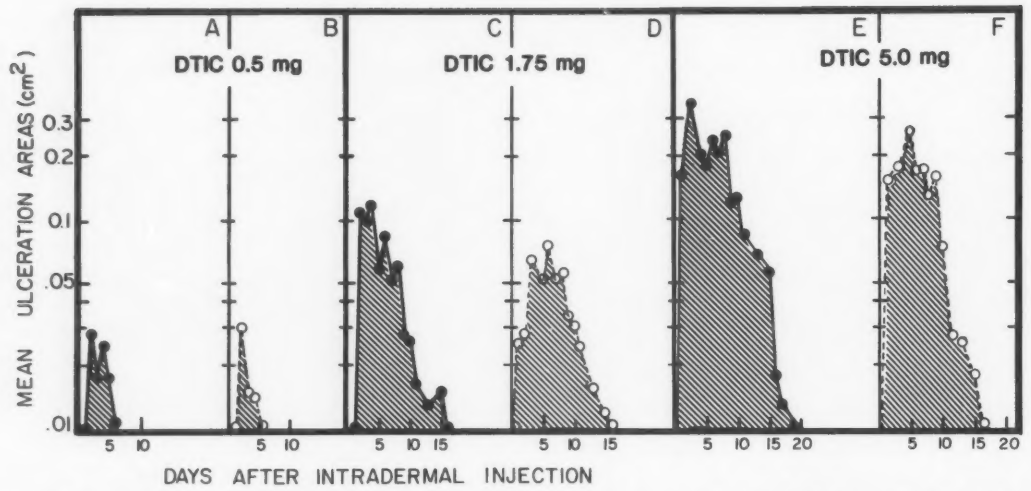


FIGURE 2.—Comparison of mean skin ulceration (log scale) over time in days of mice ($n = 5$ each) given 1 of 3 intradermal injections of DTIC: 0.5 mg (A + B), 1.75 mg (C + D), or 5.0 mg (E + F). Solid circles and lines indicate the use of light-protected DTIC solutions. Open circles and dashed lines indicate the use of light-exposed DTIC solutions.

strated that the cells and drug were receiving approximately 0.8 J/m²/second of 254-nm shortwave ultraviolet energy. Other wavelengths were not monitored but a complete white light spectrum was presented to the cells and drug.

Skin Toxicity Studies

Figure 2 and table 2 show the mean skin toxicity patterns for animals given three intradermal DTIC doses. At 0.5 mg of DTIC intradermally [fig 2A (light-protected) and 2B (light-exposed)], there was only minimal ulceration which resolved completely within 5 days of injection. The 1.75-mg intradermal dose caused substantially greater peak ulcerations; 0.148 cm² for the light-protected group (fig 2C) and 0.085 cm² for the light-exposed group (fig 2D). The 5.0-mg intradermal dose produced comparable peak ulcers in both groups. Thus, there were no statistically significant differences in ulceration patterns for any light-exposed or -protected DTIC treatments.

In contrast, when mice were exposed to light for 24 hours after injection, systemic toxicity was significantly increased (table 2). At the 10-mg intradermal DTIC dose, 60% of the mice exposed to light after injection died compared to none in the light-protected group.

Skin Toxicity Antidote Studies

A large series of potential local DTIC antidotes were evaluated against 5.0- and 10.0-mg intradermal DTIC injections. Table 3 shows the data for these studies. Of the pharmacologic antidotes tested, only microsomal enzyme exposure significantly reduced DTIC skin ulcer areas under the curve (AUCs). Peak ulcer sizes were reduced by the sulfur nucleophile sodium thiosulfate at twice normal serum osmolarity (0.3 M, table 3). In contrast, topical cooling of the DTIC 10-mg injection sites increased ulceration ($P < 0.05$). Topical heating, DMSO, L-cysteine, hyaluronidase, hydrocortisone, 0.17 M sodium thiosulfate, and 0.89% sodium chloride were also ineffective as local remedies to DTIC.

DISCUSSION

Light-enhanced cytotoxicity with DTIC was initially described in cultures of *Bacillus subtilis* (13). A similar pattern has also been seen in vitro with CHO cells (14)

TABLE 2.—Mean skin ulceration data for DTIC light/dark studies*

DTIC dose (mg)	DTIC light exposure†	AUC (cm² days)	Peak lesion size (cm²)	Duration of ulceration (days)
0.5	+V	0.09 ± 0.06	0.03 ± 0.02	5.0 ± 3.7
0.5	−V	0.1 ± 0.21	0.04 ± 0.02	4.2 ± 5.3
1.75	+V	0.55 ± 0.24	0.09 ± 0.03	14.8 ± 3.1
1.75	−V	0.76 ± 0.29	0.15 ± 0.05	13.0 ± 1.7
5.0	+V	1.53 ± 0.27	0.26 ± 0.09	15.0 ± 0.8
5.0	−V	2.26 ± 0.79	0.31 ± 0.12	16.8 ± 0.5
5.0	+M	3.01 ± 0.16	0.47 ± 0.19	16.0 ± 4.0
5.0	−M	1.97 ± 1.38	0.34 ± 0.17	10.0 ± 4.5
10.0	+M	1.85 ± 1.68‡	0.33 ± 0.30‡	3.8 ± 4.9‡
10.0	−M	4.81 ± 1.59	0.64 ± 0.16	14.2 ± 1.9

*Values = mean ± SD.

†+ = light-exposed; − = light-protected. V = vial of DTIC solution exposed to light for 1 hr prior to injection; M = mouse exposed to light for 24 hrs after intradermal injection with freshly prepared DTIC.

‡Low values due to early death of 6 of 10 mice.

TABLE 3.—Comparison of DTIC skin ulceration patterns*

Local adjuvant treatment (dose)	Ulceration AUC in cm^2 days at intradermal DTIC dose		Peak skin ulcer size in cm^2 at intradermal DTIC dose		Duration of ulcer in days at intradermal DTIC dose	
	5.0 mg	10.0 mg	5.0 mg	10.0 mg	5.0 mg	10.0 mg
Control	1.65 ± 1.2	3.45 ± 1.35	0.31 ± 0.11	0.81 ± 0.73	9.2 ± 5.4	13.5 ± 2.0
L-Cysteine (0.05 mol intradermally)	3.28 ± 0.85	4.77 ± 3.91	0.88 ± 0.38	1.61 ± 0.39	19.0 ± 6.3	14.2 ± 8.4
DMSO (100%)†	1.25 ± 1.2	3.19 ± 2.06	0.48 ± 0.34	1.51 ± 0.36	18.2 ± 11.5	19.0 ± 3.2
Hyaluronidase (10 units intradermally)	1.38 ± 1.1	4.19 ± 1.02	0.52 ± 0.35	2.65 ± 0.85‡	13.4 ± 8.0	20.8 ± 6.3
Hydrocortisone (2.5 mg intradermally as sodium succinate salt)	2.15 ± 1.1	3.28 ± 2.08	0.37 ± 0.12	1.64 ± 1.01	9.8 ± 3.9	22.8 ± 3.8
Microsomal enzyme/NADPH pre-exposure	0.23 ± 0.41‡	1.66 ± 1.37‡	0.05 ± 0.06‡	0.23 ± 0.32‡	3.4 ± 3.1‡	6.4 ± 8.8
Sodium chloride (0.05-ml volume dilution, control)	1.96 ± 0.46	4.93 ± 3.02	0.33 ± 0.31	1.59 ± 1.09	14.4 ± 3.2	26.6 ± 5.7
Sodium thiosulfate						
0.17 M	1.34 ± 1.23	2.24 ± 0.76	0.33 ± 0.40	1.07 ± 1.2	11.4 ± 9.0	18.0 ± 3.9
0.33 M	0.93 ± 0.46	ND	0.12 ± 0.12‡	ND	5.2 ± 4.8	ND
Topical temperature modification for 30 mins						
Cold (8°C–10°C)	1.51 ± 0.65	9.51 ± 8.11‡	0.38 ± 0.25	3.80 ± 2.28‡	16.2 ± 7.1	27.0 ± 4.7
Heat (43°C–44°C)	1.06 ± 1.18	6.95 ± 7.75	0.23 ± 0.25	0.89 ± 0.82	7.6 ± 4.7	10.0 ± 7.7

*Values = mean ± SD. ND = treatment not done.
†2 topical applications.
‡Indicates a statistically significant treatment difference by SNK multiple range test.

and with L1210 cells (15). In the current study, these two mammalian cell lines did not demonstrate light-enhanced DTIC cytotoxicity with either a 1-hour preincubation light exposure or with a concurrent 1–6-hour drug and light incubation in vitro. In our experiments, a consistent DTIC dose response was obtained following exposure to relatively high concentrations of drug. Thus, both cell lines are relatively resistant to DTIC in vitro. This probably results from the requirement for microsomal activation of DTIC to develop maximal antitumor activity (16). This activation step is believed to involve oxidative *N*-demethylation to yield monomethyl-5-aminoimidazole-4-carboxamide (MTIC) and formaldehyde (17,18). The ultimate toxic intermediate from this requisite metabolism appears to be a highly reactive methyldiazonium ion, $CH_3N^+ \equiv N$, which can readily methylate nucleic acids in DNA (9,16,18). Alkali-labile lesions in the DNA are also produced by the MTIC metabolite and may relate to pH effects on apurinic sites in DNA undergoing repair (19).

Previous studies have shown the transplantable, chemically induced 07/A mouse colon tumor to be markedly responsive to DTIC (20). In the current study, DTIC significantly inhibited 07/A colon tumor growth in mice but pre-exposure of drug to light did not increase this antitumor activity.

Other in vitro studies with CHO cells have shown time-dependent enhancement of DTIC cytotoxicity with concurrent light exposure (14). Maximal light enhancement was achieved at 12 hours but was nonetheless substantial with 6 hours of light and drug exposure. The present DTIC results with CHO cells did not duplicate these light-enhanced cytotoxicity patterns. Like the results with CHO cells, we were also unable to enhance DTIC-induced L1210 cytotoxicity by light exposure. The current results are, however, consistent with recent metabolic studies. Mizuno and Decker (19) have shown that DTIC, which is photodecomposed to 2-azahypoxanthine, does not produce DNA methylation in vitro. In addition, the chemical nucleophile 4-nitrobenzylpyridine does not react with either parent DTIC or 2-azahypoxanthine, while the MTIC metabolite is reactive. This suggests that neither parent drug nor the photodecomposed product has DNA-alkylating activity. Our in vitro antitumor studies support this hypothesis.

Clinically, it has been suggested that acute DTIC toxic effects are enhanced if the drug is exposed to light during preparation and/or administration (4). Light protection of DTIC solutions during administration has conversely been associated with reduced nausea and vomiting and local venous pain in one preliminary report (21). Thus, scrupulous light protection for all DTIC solutions used clinically is currently recommended (22). Other clinical reports describe DTIC photosensitivity in patients receiving moderate- (5) to high-dose therapy (2,3). Beck et al (5) described two cases of stinging and erythema on sun-exposed skin several hours after patients received DTIC. The reaction, which involved the hand of injection and the face, worsened with sunlight exposure and subsided spontaneously 3–4 days after

stopping therapy. In a recent phase I trial of intermittent high-dose DTIC, Buesa et al (23) described intense sunlight-induced photosensitivity in seven of 26 (27%) patients receiving doses of 1380–1980 mg/m^2. Again, the reaction typically involved the head and hands, began immediately after injection, and abated upon moving to shady areas. Because of these reports, we examined DTIC skin toxicity in a quantitative murine skin toxicity model. Our intradermal DTIC studies show that very high concentrations of drug are required to produce dose-dependent skin ulcers in mice. The results showed that light-exposure of DTIC produces increased skin toxicity in the mouse. In contrast, when DTIC-treated mice were then housed in the light or dark, a significant difference emerged: six of ten light-exposed mice were killed compared to none of ten light-protected mice. All injections (time of day, strain, gender, etc) were identical. This finding is consistent with clinical experience with this drug and suggests that sunlight exposure should be avoided in patients for several days following DTIC administration.

Local DTIC antidote studies in BALB/c mice also showed that a variety of potential local antidotes were not effective at reducing DTIC skin ulcers. Topical skin site cooling to 8°C–10°C for 30 minutes significantly enhanced skin ulceration and should be contraindicated for clinical use. The most effective DTIC skin toxicity antidote proved to be 0.33 M sodium thiosulfate. However, clinical observations of serious DTIC extravasation reactions have not been reported; thus, the use of thiosulfate as a local antidote should be reserved for only those situations in which a large amount of drug has infiltrated outside of the vein.

In summary, the current results suggest that DTIC solutions do not require elaborate protection from light during administration to patients. Conversely, patients receiving DTIC *do* require protection from direct sunlight during the 3–5-day period following drug injection. This simple precaution may prevent severe DTIC photosensitivity reactions. A role for light in enhancing DTIC antitumor effects in vivo and in vitro was not established in this study.

REFERENCES

1. MONTGOMERY JA. Experimental studies at Southern Research Institute with DTIC (NSC-45388). Cancer Treat Rep 60:125–134, 1976.
2. BEAL DD, SKIBBA JL, WHITNABLE KK, ET AL. Effects of 5-(3,3-dimethyl-1-triazeno) imidazole-4-carboxamide and its metabolites on Novikoff hepatoma cells. Cancer Res 36:2827–2831, 1976.
3. SCHABEL FM. Single-agent treatment of ten transplantable colon tumors of mice in Southern Research Institute. Annual Report to the Division of Cancer Treatment, National Cancer Institute, Section 11, 1981, p 29.
4. BAIRD GM, and WILLOUGHBY MLN. Photodegradation of dacarbazine. Lancet ii:681, 1978.
5. BECK TM, HART NE, and SMITH CE. Photosensitivity reaction following DTIC administration: report of two cases. Cancer Treat Rep 64:725–726, 1980.
6. DORR RT, ALBERTS DS, and CHEN H-SG. Experimental model of doxorubicin extravasation in the mouse. J Pharmacol Methods 4: 237–250, 1980.
7. DORR RT, ALBERTS DS, and STONE A. Cold protection and heat enhancement of doxorubicin skin toxicity in the mouse. Cancer Treat Rep 69:431–437, 1985.
8. AMES BN, MCCANN JK, and YAMASAKI E. Methods for detecting carcinogens and mutagens with the salmonella mammalian-microsome mutagenicity test. Mutat Res 31:347–359, 1975.
9. SAUNDERS PP, and CHAO LY. Fate of the ring moiety of 5-(3,3-dimethyl-1-triazeno) imidazole-4-carboxamide in mammalian cells. Cancer Res 34:2464–2469, 1974.
10. FREIREICH EJ, GEHAN EA, RALL DP, ET AL. Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey, and man. Cancer Chemother Rep 50:219–244, 1966.
11. GEHAN EA. A generalized Wilcoxon test for comparing arbitrarily singly-censored samples. Biometrika 52:203–210, 1965.
12. MANTEL N. Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer Chemother Rep 50: 163–170, 1966.
13. SAUNDERS PP, and SCHULTZ GA. Studies of the mechanism of action of the antitumor agent 5(4)-(3,3-dimethyl-1-triazeno)-imidazole-4(5)-carboxamide in *Bacillus subtilis*. Biochem Pharmacol 19: 911–919, 1970.
14. GERULATH H, BARRANCO SC, and HUMPHREY RM. The effects of treatments with (3,3-dimethyl-1-triazeno) imidazole-4-carboxamide in darkness and in light on survival and progression of Chinese hamster ovary cells in vitro. Cancer Res 34:1921–1925, 1974.
15. METELMANN H-R, and VON HOFF DD. In vitro activation of dacarbazine (DTIC) for a human tumor cloning system. Int J Cell Cloning 1:24–32, 1983.
16. LOO TL, HOUSHOLDER GE, GERULATH AH, ET AL. Mechanism of action and pharmacology studies with DTIC (NSC-45388). Cancer Treat Rep 60:149–152, 1976.
17. SKIBBA JL, BEAL DP, and RAMIREZ G. *N*-Demethylation of the antineoplastic agent 4(5)-(3,3-dimethyl-1-triazeno) imidazole-5(4)-carboxamide by rats and man. Cancer Res 30:147–150, 1970.
18. MIZUNO NS, and HUMPHREY EW. Metabolism of 5-(3,3-dimethyl-1-triazeno) imidazole-4-carboxamide (NSC-45388) in human and animal tumor tissue. Cancer Chemother Rep 56:465–472, 1972.
19. MIZUNO NS, and DECKER RW. Alteration of DNA by 5-(3-methyl-1-triazeno) imidazole-4-carboxamide (NSC-407347). Biochem Pharmacol 25:2643–2647, 1976.
20. GRISWOLD DP, CORBETT TH, and SCHABEL FM. Experimental tumor models for evaluation of imidazole-4-carboxamide, 5-(3,3-dimethyl-1-triazino) (DTIC), NSC-45388. Proc Am Assoc Cancer Res and ASCO 22:205, 1981.
21. KORIECH O, and SHUKLA V. Reduced toxicity of DTIC with administration in the dark. Proc Am Assoc Cancer Res and ASCO 21:168, 1980.
22. SHUKLA VS. A device to prevent photodegradation of dacarbazine (DTIC). Clin Radiol 31:239–240, 1980.
23. BUESA JM, GRACIA M, VALLE M, ET AL. Phase I trial of high-dose dacarbazine. Cancer Treat Rep 68:499–504, 1984.

Phase I Study of Epirubicin Given on a Weekly Schedule[1,2]

R. Snyder,[3,*] J. Bishop,[4] G. Brodie,[5] W. Burns,[3] A. Coates,[6] J. Levi,[7] D. Raghavan,[8] M. Schwarz,[9] M. Tattersall,[6] D. Thomson,[10] R. Woods,[7] and V. Ziogas[11]

Epirubicin was studied in a phase I setting to find the maximum tolerated dose when given weekly for 3 of 4 weeks. Forty-one evaluable patients were treated in groups at doses increasing from 20 to 45 mg/m^2. The highest dose level produced the maximum degree of myelosuppression (lowest neutrophil count, 1.9×10^9/L; range, 0–3.7) recorded on Day 22. This was well-tolerated in this group of mainly pretreated patients. Nonhematologic side effects were minimal. This dose schedule allows a greater dose per unit time to be administered than other recommended schedules for epirubicin. [Cancer Treat Rep 71:273–276, 1987]

Epirubicin is a stereoisomer of doxorubicin which is being investigated because initial studies (1) suggest that it is less toxic than the parent compound while retaining its antitumor activity. The current study is a phase I study evaluating the maximum tolerated dose and toxicity of the drug when given on a weekly schedule.

Studies have shown that epirubicin binds less avidly to DNA than doxorubicin and has lower alkalinity which may give rise to higher concentrations of the active form within tumor cells than is seen with doxorubicin. Both drugs are equally effective in inhibiting nucleic acid polymerases and in preventing in vivo nucleic acid synthesis. Both drugs (1) have similar activity against Sarcoma 180, Gross leukemia, L1210 and P388 leukemias, and C3H/He mammary carcinoma. Epirubicin is more effective against Lewis carcinoma, MS-2 sarcoma, and MSV-induced rhabdomyosarcoma. Acute and chronic toxicity studies in various animal species (2) show effects qualitatively similar but generally quantitatively less than with doxorubicin.

In phase I studies utilizing 3-week schedules (3,4), epirubicin appears to cause less myelosuppression than doxorubicin when given at equimolar doses. In part, this may be explained by its terminal half-life of 30–40 hours compared to 40–70 hours for doxorubicin (5). The weekly schedule was evaluated since there are some data with doxorubicin (6–9) to suggest that this schedule may be less cardiotoxic and have less acute toxicity than the conventional 3-week schedule. Since epirubicin appears to have less toxicity than doxorubicin overall, a weekly schedule might allow a larger dose per unit time to be given. At the time this trial started, the recommended dose of epirubicin was 60–90 mg/m^2 every 3 weeks (3,4). The starting dose of 20 mg/m^2 for 3 of 4 weeks is equivalent to 45 mg/m^2 every 3 weeks and was thought to be tolerable.

PATIENTS AND METHODS

Patients were eligible if they had a histologically confirmed diagnosis of malignancy which was either resistant to conventional therapy or lacked standard therapy; performance status of 0–3 [Eastern Cooperative Oncology Group (ECOG) scale]; no chemotherapy or radiotherapy within 3 weeks (6 weeks for nitrosoureas) and recovered from the toxicity of any prior therapy; and pretreatment leukocyte count $> 3 \times 10^9$/L, platelet count $> 125 \times 10^9$/L, creatinine < 0.150 mmol/L, and bilirubin < 25 μmols/L (unless due to hepatic metastases < 45 μmols/L). Patients were ineligible if they had a history of pre-existing cardiac disease or if a gated cardiac pool scan was abnormal after receiving ≥ 300 mg/m^2 of doxorubicin. This was a multi-institutional study accruing patients from seven different hospitals. Informed consent was obtained from each patient ac-

[1] Received May 19, 1986; revised Oct 31, 1986; accepted Nov 20, 1986.
[2] Supported by Farmitalia Carlo Erba.
[3] St. Vincent's Hospital, Fitzroy, Victoria, Australia.
[4] Peter MacCallum Hospital, Melbourne, Victoria, Australia.
[5] Prince Henry's Hospital, Melbourne, Victoria, Australia.
[6] Ludwig Institute for Cancer Research, Sydney, New South Wales, Australia.
[7] Royal North Shore Hospital, St. Leonards, New South Wales, Australia.
[8] Royal Prince Alfred Hospital, Camperdown, New South Wales, Australia.
[9] Alfred Hospital, Prahran, Victoria, Australia.
[10] Princess Alexandra Hospital, Woolloongabba, Queensland, Australia.
[11] COSA Cancer Trials Data Centre, Parkville, Victoria, Australia.
* *Reprint requests to:* Dr. R. D. Snyder, Oncology Unit, St. Vincent's Hospital, Victoria Parade, Fitzroy, Victoria 3065, Australia.

cording to the policy of each institutional ethics committee.

Pretreatment assessment consisted of history and physical examination, tumor measurement, cbc, assay for serum alkaline phosphatase, aspartate aminotransferase, bilirubin, creatinine, urea, albumin, and total protein. ECG and chest x-ray were performed before treatment as were other radiological and nuclear scan examinations, as clinically indicated. During therapy patients had weekly cbc and at a minimum four weekly liver and renal function tests unless indicated more frequently. In fact, 25 patients had liver and renal function tests performed at least every 2 weeks. Cardiologic assessment including gated cardiac pool scans was required when the total dose of anthracyclines reached 300 mg/m^2 and monthly thereafter or if there was clinical evidence of cardiac toxicity.

Epirubicin was supplied by Farmitalia Carlo Erba in vials containing 10 or 50 mg as a red lyophilized powder. The drug was given on Days 1, 8, and 15 with the next course starting on Day 28.

A total of 47 patients were entered (21 male; 26 female), with a median age of 57 years (range, 21–77). One patient was inevaluable since no treatment with epirubicin was given. Four patients had had no prior chemotherapy. Of 42 previously treated patients, 21 had received doxorubicin (median dose, 280 mg/m^2; range, 70–540). The performance status was ECOG grade 0 (four patients), 1 (19 patients), 2 (12 patients), and 3 (11 patients). The main tumor types were soft tissue sarcoma (ten patients), breast carcinoma (seven), non-small cell lung carcinoma (five), and small cell lung, ovarian, kidney, and head and neck carcinomas (four each).

The dose level of epirubicin received was 20 mg/m^2 (five evaluable patients), 30 mg/m^2 (eight patients), 35 mg/m^2 (11 patients), 40 mg/m^2 (six patients), and 45 mg/m^2 (11 patients). The dose was not escalated in individual patients. The dose was escalated after a minimum of four evaluable patients had completed their treatment at each dose level. Patients were evaluable for cumulative toxicity (in particular, myelosuppression) if they received at least two doses of the drug 1 week apart. All doses were evaluated for acute (nonhematologic) toxicity. Five patients were inevaluable since they failed to receive the minimum number of doses despite the absence of toxicity. Three patients who were technically ineligible because of increased serum creatinine levels (0.15–0.20 μmol/L) were evaluated. The protocol required withholding treatment if grade 3 or 4 toxicity (10) developed, and subsequent doses were reduced by one dose level on recovery from grade 4 toxicity. Patients were removed from the study if they developed progressive disease after the first or subsequent courses. The number of courses evaluated at each dose level was seven at 20 mg/m^2, 14 at 30 mg/m^2, 16 at 35 mg/m^2, eight at 40 mg/m^2, and 14 at 45 mg/m^2.

RESULTS

The details of myelosuppression are shown in table 1. At the maximum dose level, the median lowest neutrophil count was 1.9×10^9/L, with three of 11 patients having $< 1.0 \times 10^9$/L (grade 3 or 4 toxic effects). In each of these patients the counts had fallen by Day 15 and that dose was omitted; it recovered again by Day 28. The effect on platelet counts was mild (no counts $< 110 \times 10^9$/L) and on hemoglobin was negligible. The non-

TABLE 1.—Summary of leukocyte and neutrophil count data

Blood cell count*	Dose level (mg/m^2)				
	20	30	35	40	45
	Day 1				
Mean wbc count	7.2	8.7	6.4	6.6	9.1
Range	6.9–9.6	4.8–29	2.7–39	3.4–29	3.2–22
Mean neutrophil count	4.8	6.1	3.7	7.9	6.2
Range	4.1–7.5	3.6–25	1.8–15	2.7–24	2.5–17
	Day 8				
Mean wbc count	6.2	8.9	4.8	15.1	8.2
Range	3.8–9.8	5.2–42	3.4–11.8	0.5–21	2.9–27
Mean neutrophil count	4.45	6.2	4.7	9.4	7.5
Range	2.0–7.9	3.9–39	2.6–11.5	0.4–19	2.6–23
	Day 15				
Mean wbc count	6.5	7.9	3.3	9.2	3.7
Range	4.3–10.2	3.4–38	1.5–8.2	3.3–14	1.1–18
Mean neutrophil count	5.1	5.5	3.2	4.8	3.2
Range	2.7–7.6	1.6–35	0.9–7.5	3.1–11	0.9–14
	Day 22				
Mean wbc count	7.5	16.0	3.0	2.0	2.9
Range	6.2–8.1	3.7–29	1.8–4.7	0.9–4.3	0.5–4.3
Mean neutrophil count	5.2	15.2	2.0	1.2	1.9
Range	4.2–5.3	2.5–23	1.0–4.7	0.5–2.9	0–3.7

*$\times 10^9$/L.

TABLE 2.—Summary of nonhematologic toxicity

Dose level (mg/m^2)	No. of doses	Grade*	Toxic effect				
			Stomatitis	Vomiting/ nausea	Bowel	Alopecia	Infection
20	19	1	—	—	—	—	—
		2	—	3	—	—	2
		3	—	—	—	—	—
30	46	1	—	11	—	—	—
		2	—	3	—	—	—
		3	—	1	—	—	—
35	42	1	—	5	1	—	—
		2	—	20	—	3	—
		3	—	—	—	—	—
40	25	1	—	16	—	3	—
		2	—	2	—	1	—
		3	—	—	—	—	—
45	44	1	1	8	1	2	—
		2	—	2	—	—	—
		3	—	—	—	—	—

*See ref 10.

hematologic toxicity for all doses administered is summarized in table 2.

One case of probable cardiac toxicity was noted. A 45-year-old female who had previously received 540 mg/m^2 of doxorubicin had a fall in ejection fraction on serial gated cardiac pool scans from 62% to 47% after 225 mg/m^2 of epirubicin without clinical signs of cardiac failure.

Thirty patients were removed from the study because of progressive disease, and another seven died in the study without evidence of toxicity (average, three doses; average cumulative dose, 115 mg/m^2). Three patients refused further treatment (unrelated to toxicity in two patients and associated with unacceptable anorexia and malaise in a third). Three patients had grade 4 neutropenia and were withdrawn. No treatment-related deaths were observed. Although more frequent administration of the drug might be expected to lead to an increase in the incidence of extravasation, none was noted. Two partial responses were achieved, one in a patient with breast carcinoma who had previously responded to doxorubicin (who received 35 mg/m^2 of epirubicin) and one in an anthracycline-naive patient with small cell lung carcinoma (who received 30 mg/m^2 of epirubicin). These responses lasted < 2 months.

DISCUSSION

Epirubicin is a new anthracycline which is reported to be quantitatively different from doxorubicin with respect to toxicity. Most studies (11–13) have investigated a 3-week schedule with a recommended dose of 75–90 mg/m^2 as a single agent. These studies, and some using multidrug combinations, have used a dose of epirubicin which is equimolar to doxorubicin rather than an equitoxic (and therefore larger) dose. Two previous studies have looked at a weekly schedule using doses of 12.5 (14) and 20 (15) mg/m^2, respectively. However, both of these studies report minimal toxicity and were not designed to investigate the tolerance of higher doses. This study has shown that a dose of 45 mg/m^2 can be given for 3 of 4 weeks to pretreated patients with tolerable toxicity. Further increases in dose in previously treated patients are unlikely because of myelosuppression. Too few previously untreated patients have been investigated in the current study to establish whether or not they could tolerate even higher doses.

This dose schedule is equivalent to 100 mg/m^2 on a 3-week cycle. If there is a dose-response relationship, then this new schedule may lead to an increase in effectiveness in the agent. We are currently investigating this schedule in a phase II study, looking not only at efficacy but also at its chronic toxicity, especially cardiotoxicity.

REFERENCES

1. CASAZZA AM, DI MARCO A, BONADONNA G, ET AL. Effects of modifications in position 4 of the chromophore or in position 4′ of the aminosugar, on the antitumor activity and toxicity of daunorubicin and doxorubicin. *In* Anthracyclines: Current Status and New Developments (Crooke ST, and Reich SD, eds). New York, Academic Press, 1980, pp 403–439.
2. CASAZZA AM, DI MARCO A, BERTAZZOLI C, ET AL. Antitumor activity, toxicity and pharmacological properties of 4′-epi-adriamycin. *In* Current Chemotherapy (Siegenthaler W, and Luthy R, eds). Washington, DC, American Society for Microbiology, 1978, pp 1257–1260.
3. BONFANTE V, BONADONNA G, VILLANI F, ET AL. Preliminary phase I study of 4′-epi-adriamycin. Cancer Treat Rep 63:915–918, 1979.
4. SCHAUER PK, WITTES RE, GRALLA RJ, ET AL. A phase I trial of 4′-epi-doxorubicin. Cancer Clin Trials 4:433–437, 1981.
5. NATALE N, PIAZZA E, ITALIA C, ET AL. The kinetics of anthracyclines in human plasma and tissues: daunomycin, doxorubicin and 4′-epidoxorubicin. Drug Exp Clin Res 9:775–779, 1983.

6. WEISS AJ, METTER GE, FLETCHER WS, ET AL. Studies on adriamycin using a weekly regimen demonstrating its clinical effectiveness and lack of cardiac toxicity. Cancer Treat Rep 60:813–822, 1976.
7. VON HOFF DD, LAYARD MW, BASA P, ET AL. Risk factors for doxorubicin-induced congestive heart failure. Ann Intern Med 91: 710–717, 1979.
8. CHLEBOWSKI RT, PAROLY WS, PUGH RP, ET AL. Adriamycin given as a weekly schedule without a loading course: clinically effective with reduced incidence of cardiotoxicity. Cancer Treat Rep 64:47–51, 1980.
9. TORTI FM, ASTON D, LUM BL, ET AL. Weekly doxorubicin in endocrine-refractory carcinoma of the prostate. J Clin Oncol 1:477–482, 1983.
10. MILLER AB, HOOGSTRATEN B, STAQUET M, ET AL. Reporting results of cancer treatment. Cancer 47:207–214, 1981.
11. HURTELOUP P, CAPPELAERE P, ARMAND JP, ET AL. Phase II clinical evaluation of 4′-epi-doxorubicin. Cancer Treat Rep 67:337–341, 1983.
12. FERRAZZI E, NICOLETTO O, VINANTE O, ET AL. Phase II study of 4′-epi-doxorubicin. Tumori 68:431–435, 1982.
13. CERSOSIMO RJ, and HONG WK. Epirubicin: a review of the pharmacology, clinical activity, and adverse effects of an adriamycin analogue. J Clin Oncol 4:425–439, 1986.
14. BERETTA G, TABIADON D, CLERICI M, ET AL. Clinical evaluation of 4′-epi-adriamycin in a new therapeutic regimen. *In* Proceedings of the Second European Conference on Clinical Oncology, 1983.
15. JONES WG, and MATTSSON W. Phase II study of weekly low-dose 4′-epi-doxorubicin in advanced postmenopausal breast cancer. Cancer Treat Rep 68:675–677, 1984.

Second-Line Chemotherapy of Stage III–IV Ovarian Carcinoma: A Randomized Comparison of Melphalan to Melphalan and Hexamethylmelamine in Patients With Persistent Disease After Doxorubicin and Cisplatin[1,2]

Joseph L. Pater,[3,*] John A. Carmichael,[3] Garry V. Krepart,[4] Robert C. Fraser,[5] Michel Roy,[6] Mary Ellen Kirk,[7] Martin Levitt,[4] Leigh B. Brown,[8] Kenneth S. Wilson,[9] Wendy E. Shelley,[3] and Andrew R. Willan[3,10]

A total of 205 women with stage III or IV ovarian cancer who had persistent disease after initial treatment with doxorubicin and cisplatin were randomized to receive melphalan (8 mg/m^2 orally for 4 days) or the combination of melphalan (6 mg/m^2 for 4 days) and hexamethylmelamine (120 mg/m^2 for 14 days) every 4 weeks. Only one of 64 patients with measurable disease had an objective response. The major determinants of survival after randomization were the amount of residual disease after initial chemotherapy and the type of response to initial chemotherapy. There was no overall difference in survival between the two chemotherapy regimens, but the small group of patients whose disease progressed on initial chemotherapy survived significantly longer when treated with the two-drug combination. Neither of these regimens provided effective therapy for women whose disease was not eliminated by first-line treatment. However, the superior results obtained in one subgroup with the addition of hexamethylmelamine suggest that the place of this agent in treating ovarian cancer should be carefully evaluated. [Cancer Treat Rep 71:277–281, 1987]

Since the introduction of the CHAP (cyclophosphamide, hexamethylmelamine, doxorubicin, and cisplatin) regimen (1), cisplatin-containing combination chemotherapy has become standard treatment for patients with stage III and IV ovarian carcinoma (2). However, despite the high clinical response rates achieved by cisplatin combinations, the large majority of patients so treated eventually develop progressive disease and die of their malignancies. There is, thus, a great need for effective second-line therapy.

Unfortunately, while it is known that cisplatin and doxorubicin have activity in patients who have failed alkylating agents (3,4), little information has been published on the efficacy of alkylating agents and the other component of the CHAP regimen, hexamethylmelamine, in the treatment of patients who have persistent disease after first-line therapy with doxorubicin and cisplatin. To obtain such information, in 1980 the Clinical Trials Group of the National Cancer Institute of Canada initiated a randomized trial with the objectives of assessing the relative effects on response and survival of melphalan and the combination of melphalan and

[1] Received July 28, 1986; revised Nov 6, 1986; accepted Nov 20, 1986.
[2] Supported by the National Cancer Institute of Canada.
[3] Queen's University, Kingston, Ontario, Canada.
[4] Manitoba Cancer Foundation, Winnipeg, Canada.
[5] Dalhousie University, Halifax, Nova Scotia, Canada.
[6] Laval University, Quebec, Canada.
[7] University of Western Ontario, London, Canada.
[8] University of Alberta, Edmonton, Canada.
[9] Victoria Cancer Clinic, British Columbia, Canada.
[10] We would like to thank the following individuals who, in addition to the authors, contributed patients to the study: G. Armitage, Saskatoon Cancer Clinic, Saskatoon, Saskatchewan, Canada; A. D. DePetrillo, Ontario Cancer Treatment and Research Foundation, Hamilton, Canada; P. Drouin, Ontario Cancer Treatment and Research Foundation, Ottawa, Canada; M. Goodyear, Ontario Cancer Treatment and Research Foundation, Thunder Bay, Canada; H. M. Gough, Victoria Cancer Clinic, British Columbia, Canada; P. Harris, Manitoba Cancer Treatment and Research Foundation, Winnipeg, Canada; D. Klaassen, Saskatoon Cancer Clinic, Saskatchewan; J. Kotalik, Ontario Cancer Treatment and Research Foundation, Thunder Bay; J. Latreille, L'Hôtel-Dieu de Québec, Quebec, Canada; R. Lotocki, Manitoba Cancer Treatment and Research Foundation, Winnipeg, Canada; I. Maxwell, Manitoba Cancer Treatment and Research Foundation, Winnipeg; R. Nimmagadda, Saint John Regional Hospital, New Brunswick, Canada; K. I. Pritchard, Women's College Hospital, Toronto, Ontario, Canada; M. Taylor, W. W. Cross Cancer Institute, Edmonton, Alberta, Canada; M. G. Tompkins, Nova Scotia Cancer Treatment and Research Foundation, Victoria General Hospital, Halifax, Canada; W. C. Tupper, Nova Scotia Cancer Treatment and Research Foundation, Victoria General Hospital, Halifax; and S. Yoshida, Ontario Cancer Treatment and Research Foundation, Windsor, Canada.

* *Reprint requests to:* Joseph L. Pater, MD, Clinical Trials Group, National Cancer Institute of Canada, 82–84 Barrie St, Queen's University, Kingston, Ontario K7L 3N6, Canada.

TABLE 1.—Dose modification scheme

Nadir wbc count (cells/mm^3)		Wbc count (cells/mm^3)*	Dose of each drug (%)	Platelet count (cells/mm^3)*	Dose of each drug (%)
> 2000		> 3000	100	> 100,000	100
1000-2000	and	2500-3000	50	75,000-100,000	50
< 1000	and	< 2500	0	< 75,000	0

*On treatment day.

hexamethylmelamine in patients with persistent disease after initial treatment with doxorubicin and cisplatin. The results of that study are reported here.

PATIENTS AND METHODS

All patients entered in the study had histologically confirmed epithelial carcinoma of the ovary and were International Federation of Gynecology and Obstetrics stage III or IV. After initial careful surgical staging and debulking when possible, chemotherapy with doxorubicin (50 mg/m^2) and cisplatin (50 mg/m^2) was begun. Cycles were repeated every 3-4 weeks for a total of nine courses or until disease progression. Patients who were clinically free of disease after completion of chemotherapy underwent second-look laparotomies.

Candidates for the trial came from three groups, categorized according to their status after completion of initial treatment: (*a*) *NE*—those who had no clinically detectable disease at the end of primary chemotherapy, but who had positive second-look laparotomies; some of these patients had started treatment with no clinical evidence of disease; (*b*) *PR/NC*—those who had persistent clinically detectable disease at the completion of chemotherapy, but who had not shown evidence of progression; some of these patients had repeat debulking laparotomies; and (*c*) *PD*—those whose disease progressed on initial chemotherapy. After giving informed consent, patients were stratified into the three categories mentioned and randomized centrally to one of the following chemotherapy regimens: (*a*) melphalan (8 mg/m^2 orally) daily for 4 days every 4 weeks, or (*b*) melphalan (6 mg/m^2) for 4 days and hexamethylmelamine (120 mg/m^2 orally) for 14 days every 4 weeks. Pyridoxine (100 mg three times a day) was given with hexamethylmelamine. Doses were modified on the basis of nadir and treatment day neutrophil and platelet counts, according to the scheme in table 1. Chemotherapy was to be given for 12 months or until disease progression.

In patients with measurable disease at the beginning of treatment, response was defined as follows: *complete*—

TABLE 2.—Distribution of patient characteristics by regimen*

Characteristic	Melphalan	Melphalan + hexamethylmelamine	Total
Debulking surgery done			
Unknown	7 (35.0)	13 (65.0)	20
No	90 (51.1)	86 (48.9)	176
Yes	6 (66.7)	3 (33.3)	9
Response status			
Unknown	2 (66.7)	1 (33.3)	3
No evidence of disease, positive 2nd look	61 (51.7)	57 (48.3)	118
Partial response/no change	21 (45.7)	25 (54.3)	46
Progressive disease	19 (50.0)	19 (50.0)	38
Largest residual tumor diameter (cm)			
Microscopic	25 (47.2)	28 (52.8)	53
< 2	42 (54.5)	35 (45.5)	77
2-10	23 (45.1)	28 (54.9)	51
> 10	11 (50.0)	11 (50.0)	22
Nonmeasurable	2 (100.0)	0 (0.0)	2
Age (yrs)			
< 30	2 (100.0)	0 (0.0)	2
30-39	4 (66.7)	2 (33.3)	6
40-49	19 (63.3)	11 (36.7)	30
50-59	31 (44.9)	38 (55.1)	69
60-69	33 (47.8)	36 (52.2)	69
≥ 70	14 (48.3)	15 (51.7)	29

*Values = No. of patients (%).

all clinical signs of disease have disappeared for at least 3 months; *partial*—measurable lesions have decreased by at least 50% and no new lesions have appeared; *progressive disease*—development of new lesions or a 50% increase in the size of a measurable lesion; *time to disease progression*—time from allocation to the first sign of progressive disease; and *survival*—time from allocation to death.

Data from patients in the study, including copies of surgical, pathology, and radiology reports, were reviewed centrally by one of us (W. E. Shelley). Questions concerning eligibility, evaluability, and response assessment were resolved by discussion between the trial coordinator (W. E. Shelley), the investigator, and the study chairman. All pathology specimens were reviewed centrally (by M. E. Kirk).

Planned accrual to the study was 80 patients/arm. This sample size was chosen to detect a 60% increase in survival at a one-side level of 0.05 and a power of 90%. No interim analyses were performed. The impact of prognostic factors and treatment on survival was assessed by fitting a Cox's proportional hazards model. All *P* values reported are two-sided. One-sided values can be obtained by dividing by two.

RESULTS

Of 342 eligible patients registered between December 1980 and March 1984 in the initial portion of the trial, 244 reached the point where they were eligible for randomization. Thirty-six of these patients were not randomized at their or their physician's decision. Of the 208

FIGURE 1.—Survival according to maximum diameter of residual disease after completion of doxorubicin and cisplatin therapy. 0 = microscopic only; 1 = < 2 cm; 2 = 2-10 cm; and 3 = > 10 cm.

patients randomized, three were subsequently found to be ineligible on the grounds of histology (two patients) or initial stage (one). Thus, 205 patients randomized between melphalan or melphalan and hexamethylmelamine are available for analysis. Table 2 shows the characteristics of these patients. As is evident the groups were well-balanced with respect to age, diameter of residual disease at allocation, and stratification category.

Sixty-four patients had measurable disease at allocation and were, thus, assessable for response. Only one patient responded. This was a patient receiving melphalan and hexamethylmelamine who had complete disappearance of nodularity in the vagina which appeared after three cycles of doxorubicin and cisplatin. The upper 95% confidence limit for the overall response rate (one response among 64 patients) is 0.07; that for the combination arm (one response among 34 patients) is 0.15.

The combination regimen was substantially more toxic than melphalan alone. Thirteen patients had to discontinue therapy because of toxicity. All patients were on the combination arm and in each patient the agent which had to be discontinued was hexamethylmelamine. In six of these patients the treatment-limiting toxic effect was gastrointestinal; in seven it was neurological.

The effects on survival of major prognostic factors

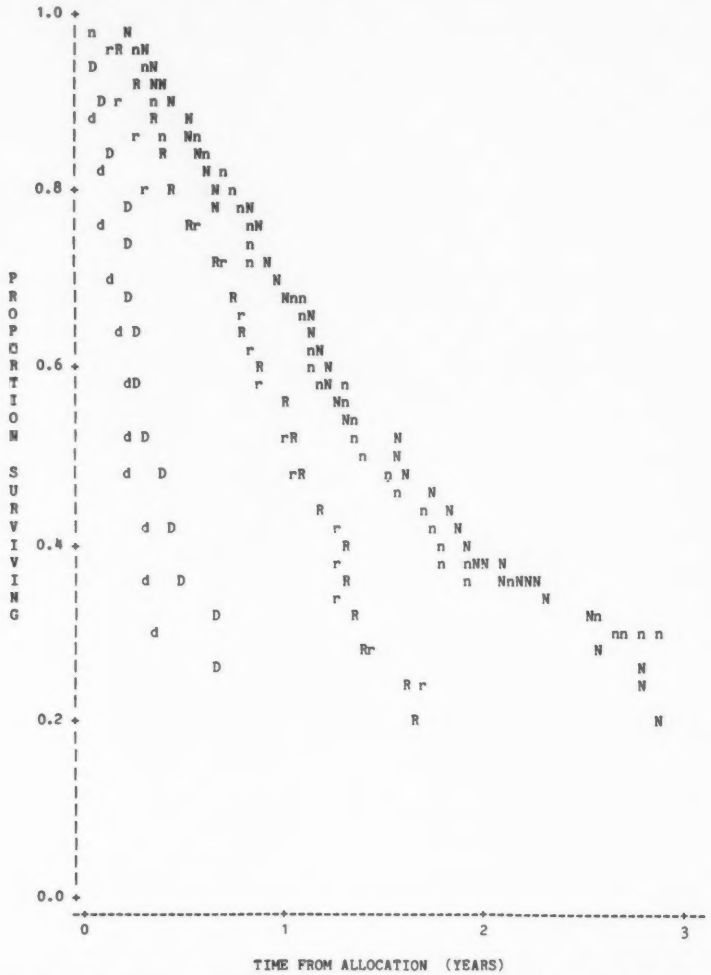


FIGURE 2.—Survival according to allocation and to response status after doxorubicin and cisplatin. Uppercase letters refer to patients allocated to melphalan and hexamethylmelamine, lowercase to those allocated to melphalan alone. Response status categories as described in the Patients and Methods section are: N,n = NE; R,r = PR/NC; and D,d = PD.

and of treatment were assessed by fitting a Cox's proportional hazards model to the data from the 200 patients on whom complete information on all prognostic factors was available. Residual tumor diameter at allocation, categorized as a continuous variable with four levels, had a major impact on survival ($P < 0.0001$). This is shown in figure 1. Disease status after initial therapy in the categories used for stratification also significantly affected survival. However, this effect was largely explained by differences in residual tumor diameter among the groups. When this latter factor was accounted for, the only disease status group whose survival was significantly different ($P < 0.0001$) was the category of patients whose disease progressed on initial chemotherapy.

There was no overall difference in survival between the two treatment groups. In addition, treatment did not have a significant effect on survival in a proportional hazards model which included the prognostic factors residual tumor diameter and disease status at allocation. However, an effect of treatment on survival was observed when an examination of treatment by prognostic factor interaction was carried out. This analysis, conducted by determining the simultaneous statistical significance of adding a single term for treatment and the two interaction terms of treatment by residual tumor diameter and treatment by disease status, produced a significant result ($P = 0.036$). This indicated that there was a significant treatment effect in at least one subgroup defined by one of the prognostic factors. Of the three terms mentioned only treatment by disease status was individually significant ($P = 0.018$), indicating that the significant treatment effect was in one of the subgroups defined by disease status at the time of allocation. An examination of survival curves for the two treatment groups in the three disease status at allocation categories (fig 2) indicates that it is the patients who progressed on doxorubicin and cisplatin who have a survival benefit from the combination regimen.

DISCUSSION

The result of this study of the most immediate clinical relevance is the fact that the fate of these patients was much more substantially affected by their status after initial therapy than by the choice of their second-line treatment. This observation supports the argument that advances in the management of patients with advanced ovarian cancer are more likely to come from the development of better initial therapy than from improved salvage regimens (5), although recent developments may alter this opinion (2).

Our findings are not entirely negative, however, in that among those patients whose disease progressed on doxorubicin and cisplatin, those treated with the combination regimen had statistically significantly longer survival. The clinical significance of this observation is obviously minimal, since the prolongation of life was very short and the addition of hexamethylmelamine produced substantial toxicity. However, there may be some biologic significance to our results since they raise the question of a potential role for hexamethylmelamine in the management of patients with ovarian cancer. At present, although hexamethylmelamine-containing combinations have shown impressive activity in some studies (1,6), the independent contribution of this agent to combination regimens has not been established (7). Indeed, in the two trials in which this question was addressed, no benefit was found to adding this drug to melphalan (8) or to doxorubicin and cisplatin (5). However, it would seem prudent to attempt to confirm our finding that at least some patients benefit from this agent before abandoning it.

Finally, it is worth noting that we have observed a significant survival benefit attributable to a regimen which produced a very low objective response rate, in circumstances where, admittedly, assessment of response is difficult. While, as noted, this finding must be interpreted cautiously, it does re-emphasize the fact that the relationship between response and survival is not always straightforward.

REFERENCES

1. GRECO FA, JULIAN CG, RICHARDSON RL, ET AL. Advanced ovarian cancer: brief intensive combination chemotherapy and second-look operation. Obstet Gynecol 58:199–205, 1981.
2. OZOLS RF. The case for combination chemotherapy in the treatment of advanced ovarian cancer. J Clin Oncol 3:1445–1447, 1985.
3. YOUNG RC, VON HOFF DD, GORMLEY P, ET AL. *cis*-Diamminedichloroplatinum(II) for the treatment of advanced ovarian cancer. Cancer Treat Rep 63:1539–1544, 1979.
4. BRUCKNER HW, RATNER LH, COHEN CJ, ET AL. Combination chemotherapy for ovarian carcinoma with cyclophosphamide, adriamycin, and *cis*-dichlorodiammineplatinum(II) after failure of initial chemotherapy. Cancer Treat Rep 62:1021–1023, 1978.
5. NEIJT JP, TEN BOKKEL HUININK WW, VAN DER BURG MEL, ET AL. Combination chemotherapy with or without hexamethylmelamine in alkylating-agent-resistant ovarian carcinoma. Cancer 53:1467–1472, 1984.
6. YOUNG RC, CHABNER BA, HUBBARD SP, ET AL. Advanced ovarian adenocarcinoma: a prospective clinical trial of melphalan (L-PAM) versus combination chemotherapy. N Engl J Med 299:1261–1266, 1978.
7. STEHMAN FB, EHRLICH CE, and CALLANGAN MF. Failure of hexamethylmelamine as salvage therapy in ovarian epithelial adenocarcinoma resistant to combination chemotherapy. Gynecol Oncol 17:189–195, 1984.
8. OMURA GA, MORROW CP, BLESSING JA, ET AL. A randomized comparison of melphalan versus melphalan plus hexamethylmelamine versus adriamycin plus cyclophosphamide in ovarian carcinoma. Cancer 51:783–789, 1983.

Chemoendocrine Therapy With Prolonged Estrogen Priming in Advanced Breast Cancer: Endocrine Pharmacokinetics and Toxicity[1,2]

Chris Benz,[3,*] David Gandara,[4] Becky Miller,[3] Thomas Drakes,[5] Scott Monroe,[6] Bruce Wilbur,[3] and Michael DeGregorio[7,8]

Recent efforts to improve response rates in advanced breast cancer have used short, alternating courses of antiestrogen therapy followed by estrogen priming to cytokinetically enhance tumor cell sensitivity to antimetabolites. Based on recent in vitro and in vivo studies, we have introduced a chemoendocrine regimen that uses prolonged courses of estrogen priming. The present protocol consists of alternating monthly cycles of tamoxifen (TAM) and estradiol during which sequential (24-hr) methotrexate, 5-fluorouracil, and leucovorin are administered at 2-week intervals. Twenty-five patients with metastatic breast cancer received > 80 endocrine cycles and > 300 courses of chemotherapy by this protocol; two-thirds of these patients had previously failed other endocrine or chemotherapy regimens. Most patients experienced grade 1 or 2 myelosuppression or gastrointestinal symptoms during at least one treatment cycle; however, overall toxicity was considered to be mild and therapy was very well tolerated. Serum levels of estradiol, estrone, TAM, and TAM metabolites were measured during all phases of the endocrine cycle in five patients on chronic therapy. Concentrations of TAM and its more abundant metabolite, *N*-desmethyltamoxifen (*N*-desTAM), rose twofold during antiestrogen therapy and fell during estrogen priming, with mean levels persisting > 100 ng/ml throughout the priming interval. Mean estradiol (E_2) and estrone (E_1) levels rose during priming and were sustained fivefold and tenfold above the basal postmenopausal levels measured during antiestrogen treatment. Calculating the serum molar ratios of [TAM + *N*-desTAM]/[E_2 + E_1] during each phase of the treatment cycle confirmed that estrogen priming was achieved pharmacologically by this endocrine schedule. Clinical remissions were observed in this small patient sample with seven of 18 patients achieving either complete (28%) or partial (11%) responses, and an additional 39% obtaining disease stabilization. Further clinical study is necessary to evaluate the optimal response rate of this regimen and to determine whether cyclic estrogen priming by this schedule results in enhanced tumor cell proliferation in vivo. [Cancer Treat Rep 71:283-289, 1987]

Complete remissions in advanced breast cancer from combination chemotherapy or endocrine therapy occur in < 20% of patients (1). Attempts to improve upon this response rate have led to many clinical trials utilizing combination chemoendocrine therapies (2). In particular, recent attention is being given to the encouraging results of estrogen priming therapy, a clinical method of enhancing breast tumor sensitivity to chemotherapeutic agents by modulating tumor cytokinetics (3–6), thereby enhancing cell cycle-specific intracellular drug activation (7–9).

Prior studies have suggested that sequential scheduling of methotrexate (MTX) and 5-fluorouracil (FUra) might improve the therapeutic index of these commonly

[1] Received July 23, 1986; revised Nov 10, 1986; accepted Nov 24, 1986.
[2] A Northern California Oncology Group study (NCOG P-1B-83-2) supported in part by Stuart Pharmaceuticals (I. C. I. Americas, Inc) and Public Health Service grants CA-36773, CA-21744 and CA-25827 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, CH-235C from the American Cancer Society, the Louis R. Lurie Foundation, and Children's Cancer Research Institute funds.
[3] Cancer Research Institute, University of California at San Francisco.
[4] Letterman Army Medical Center, Presidio of San Francisco, CA.
[5] Northern California Oncology Group Member, Redding.
[6] Department of Obstetrics and Gynecology, University of California at San Francisco.
[7] Children's Cancer Research Institute, Cancer Pharmacokinetics Laboratory, Pacific Presbyterian Medical Center, San Francisco, CA.
[8] We thank Mary Rose and Marsha Kohler for their assistance in collecting and analyzing these data.
* *Reprint requests to:* Christopher C. Benz, MD, Cancer Research Institute, M-1282, University of California at San Francisco, 1282 Moffitt Hospital, San Francisco, CA 94143.

used antimetabolites in breast cancer (10,11). Other preclinical studies suggest that combining the antiestrogen tamoxifen (TAM) with sequentially administered MTX-FUra might also lead to improved clinical responses against estrogen receptor (ER)-positive breast cancer (12). In vitro, cytostatic agents such as thymidine or TAM can be utilized to synchronize tumor cell populations (9). When such cytostatic agents are replaced by a growth-stimulating agent like estradiol (E_2), further enhancement of chemotherapeutic toxicity is observed against ER-positive tumor cells (12). Modulation of a cell population's growth fraction can be accomplished in vitro within hours by readjusting the culture concentrations of E_2 and TAM. Some studies have suggested that the parenteral administration of estrogen can enhance the proliferation of ER-positive tumor cells in vivo (13–16). However, the compounding factors of drug distribution, clearance, and metabolism for both estrogen and antiestrogen formulations complicate any repetitive attempt to modulate tumor growth and chemotherapeutic sensitivity as might be undertaken during treatment of breast cancer patients.

To date, estrogen priming studies in breast cancer patients have employed short courses of alternating antiestrogen ($\leq$ 10 days), followed by estrogen ($\leq$ 4 days) priming (3–6). The present protocol employs alternating monthly courses of TAM and E_2 priming, during which time sequential MTX-FUra is administered at 2-week intervals. These more prolonged intervals of TAM and E_2 treatment are designed to meet the pharmacologic demands of cycling these endocrine agents in vivo. In particular, the long time period (weeks) required for TAM to saturate tissue compartments, and the similar interval necessary to clear the drug and its metabolites from circulation, suggest that monthly scheduling of TAM and E_2 priming might better achieve the desired cyclical modulations in tumor growth fraction that would enhance MTX-FUra effectiveness in vivo. The objectives of the present study are both pharmacological and clinical. Specifically these include: (*a*) assessing the cyclical changes in serum concentrations of TAM, E_2, and their metabolites during all phases of the protocol treatment; (*b*) reporting on the toxicity from repeated administration of this form of combination chemoendocrine therapy; and (*c*) documenting any clinical responses observed in this treated patient population.

MATERIALS AND METHODS

Patients and Study Parameters

Premenopausal and postmenopausal patients < 76 years of age and with informed consent were eligible for treatment if they had either estrogen or progesterone receptor-positive or receptor-unknown breast carcinoma, along with measurable and biopsy-proven metastatic spread of their tumor. Those who had failed while receiving prior chemotherapy (including MTX and FUra) or endocrine treatment were considered eligible if their last course of therapy had been administered > 4 weeks prior to study entry; these patients were analyzed separately. Pretreatment and follow-up evaluations included history, physical examination, cbc, serum chemistry, and radiographs or scans as necessary. Blood count and serum creatinine were checked prior to each chemotherapy treatment. Selected patients had periodic venipunctures for determination of serum E_2 and estrone (E_1) by radioimmunoassay (17). These were collected between 2–4 hours after oral dosing with E_2, or within 6 hours following TAM ingestion. TAM and TAM metabolites were determined by high-performance liquid chromatography (HPLC) (18). Serum samples for quantitative analysis of TAM and the metabolites 4-hydroxytamoxifen (4-OHTAM) and *N*-desmethyltamoxifen (*N*-desTAM) were chemically extracted, activated to fluorescence, and simultaneously assayed by reverse-phase HPLC. The sensitivity by this assay for each metabolite is $\geq$ 8 ng/ml.

Treatment Schedule and Patient Evaluation

The treatment schedule is shown schematically in figure 1. Hormonal therapy consisted of alternating TAM and E_2 monthly. TAM (Nolvadex, generously supplied by Stuart Pharmaceuticals) was given 20 mg twice daily on Days 0 through 27 every other month. Oral E_2 (Estrace, Mead Johnson) was given 1 mg three times daily on Days 0 through 20 every other month. Chemotherapy consisted of 24-hour sequenced administrations of MTX and FUra, followed by leucovorin (supplied by Lederle Labs). This previously described outpatient chemotherapy regimen was administered on Days 6 and 20 of every 28-day cycle (11). MTX was given orally at 50 mg/m^2 every 6 hours for five doses and followed immediately by an iv bolus dose of FUra, 600 mg/m^2. Leucovorin was given orally at 10 mg/m^2 every 6 hours, for a total of six doses, beginning 6 hours after the final dose of MTX. Dose reductions (and/or schedule modifications) were made for grade 2 or worse leukopenia, thrombocytopenia, nausea, vomiting, or stomatitis; this grading system has also been described previously (19). Clinical responses were measured after a minimum of 8 weeks on treatment (one complete endocrine cycle including four courses of MTX-FUra). A complete clinical response (CR) was defined as the total disappearance of all measurable lesions with no evidence of new lesions. When a previously positive bone scan showed no progression but failed to revert completely to normal, and if all other measurable lesions disappeared entirely for at least a 2-month interval, this was considered an equivocal CR in accordance with standard Northern California Oncology Group (NCOG) reporting criteria. Partial response (PR) was defined as a $\geq$ 50% reduction in the product of two perpendicular tumor diameters without disease progression elsewhere. Stable disease was defined as a < 50% reduction in tumor volume lasting at least 2 months; and disease progression was defined as a $\geq$ 25% increase in tumor volume or the appearance of new metastatic lesions.

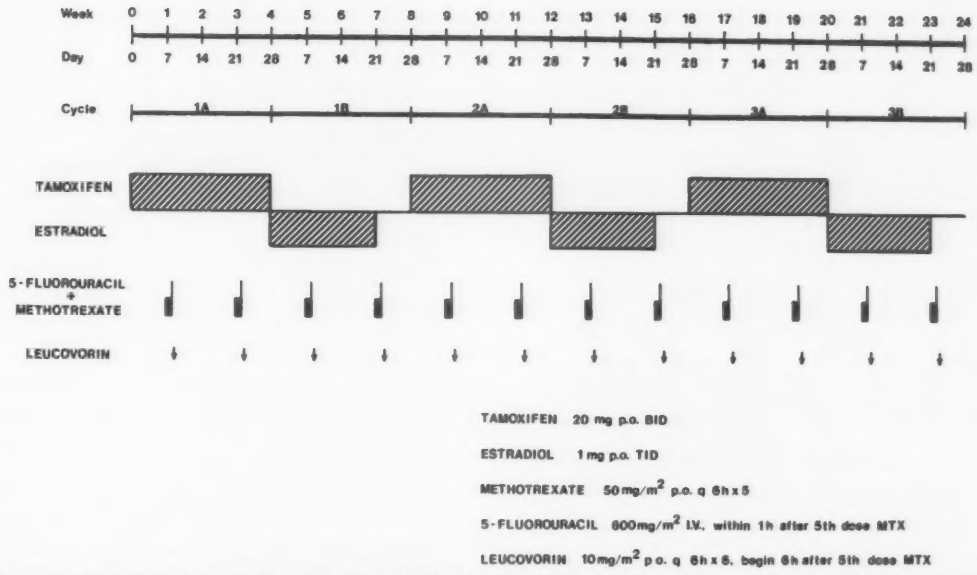

Week 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
Day 0 7 14 21 28 7 14 21 28 7 14 21 28 7 14 21 28 7 14 21 28 7 14 21 28
Cycle 1A 1B 2A 2B 3A 3B
TAMOXIFEN
ESTRADIOL
5-FLUOROURACIL + METHOTREXATE
LEUCOVORIN
TAMOXIFEN 20 mg p.o. BID
ESTRADIOL 1 mg p.o. TID
METHOTREXATE 50 mg/m2 p.o. q 6h x 5
5-FLUOROURACIL 600 mg/m2 I.V. within 1h after 5th dose MTX
LEUCOVORIN 10 mg/m2 p.o. q 6h x 6, begin 6h after 5th dose MTX

FIGURE 1.—Schematic of chemoendocrine pilot protocol (NCOG P-1B-83-2) for advanced breast cancer. Drugs and endocrine agents are given in the indicated doses and according to the above schedule for each day, week, and cycle over a 6-mo course of therapy (*see* Methods section). Response to treatment is evaluated after a minimum of 8 weeks on therapy (or 1 full endocrine cycle and 4 courses of chemotherapy).

RESULTS

A summary of characteristics for the 25 patients described in this study is shown in table 1. Nine patients received no treatment prior to entering this protocol, and the remaining 16 patients had failed prior treatment with chemotherapy and/or endocrine therapy, as shown. The combined group of patients received 81.5 cycles of endocrine (TAM $\rightarrow$ E_2) therapy and 326 courses of MTX-FUra chemotherapy.

Table 2 indicates the highest grade of hematologic and nonhematologic treatment toxicity experienced by each of the patient groups receiving the total amount of chemoendocrine therapy shown in table 1. As is evident from table 2, this therapy resulted in predominantly mild to moderate (grade 1–2) myelosuppression or gastrointestinal symptoms in approximately one-half of all treated patients. Nonhematologic toxicity was about equally distributed in both patient groups, and this consisted largely of nausea and vomiting. Myelosuppression was more than twice as prevalent in the treatment group that received prior therapy. Leukopenia occurred in only 22% of patients who received no prior therapy, and thrombocytopenia did not occur at all in this patient group.

Given the prolonged interval of estrogen priming in this regimen, it is significant that there were no occurrences of tumor flare, hypercalcemia, or dysfunctional uterine bleeding. In addition, there was no evidence of renal, hepatic, or central nervous system toxicity.

TAM serum levels were determined in two patients during the initial month of TAM administration and the following month of E_2 priming (table 3). These data suggested that TAM concentrations above the accepted therapeutic level of 100 ng/ml were being achieved during antiestrogen therapy, with lower concentrations per-

TABLE 1.—Patient characteristics*

	Prior treatment† ($N = 16$)	No prior treatment ($N = 9$)
Mean age in yrs (range)	49 (29–69)	52 (33–67)
Postmenopausal	8 (50%)	7 (78%)
Receptors		
Positive	14 (87%)	6 (67%)
Unknown	2 (13%)	3 (33%)
Metastatic sites		
Soft tissue (skin, chest wall, nodes)	11 (69%)	4 (44%)
Bone	12 (75%)	5 (56%)
Viscera (lung, pleura, liver)	9 (56%)	3 (33%)
Karnofsky		
100	0	4
90	6	1
80	9	3
70	0	1
60	1	0
Total TAM $\rightarrow$ E_2 cycles	53.5	28
Total MTX-FUra courses	214	112

*Unless otherwise indicated, values = No. of patients.

†Prior chemotherapy (10 patients) included combinations of cyclophosphamide, doxorubicin, MTX, FUra, vincristine, and mitomycin; prior endocrine therapy included bilateral salpingo-oophorectomy (3), TAM (11), megestrol acetate (4), diethylstilbestrol (2), and aminoglutethimide (1).

TABLE 2.—Toxicity

Toxic effects and grades	Prior treatment ($N = 16$)	No prior treatment ($N = 9$)
Nonhematologic		
Nausea/vomiting		
1	4	3
2	4	3
3	1	0
4	0	0
(%)	(56%)	(67%)
Diarrhea		
1	1	0
2	1	1
3	0	0
4	0	0
(%)	(13%)	(11%)
Oral mucositis		
1	1	1
2	2	1
3	0	0
4	0	0
(%)	(19%)	(22%)
Hematologic		
Wbc count ($\times 10^3$)		
1 (3.0–4.4)	2	2
2 (2.0–2.9)	4	0
3 (1.0–1.9)	2	0
4 (< 1.0)	0	0
(%)	(50%)	(22%)
Platelet count ($\times 10^3$)		
1 (90–129)	4	0
2 (50–89)	1	0
3 (25–49)	0	0
4 (< 25)	0	0
(%)	(31%)	(0%)

sisting at least a week beyond the initiation of E_2 priming. The elevated serum levels of *N*-desTAM and much lower levels of 4-OHTAM were consistent with previous reports on TAM metabolism (20–22); however, the persistence of circulating antiestrogen during estrogen priming suggested that a more detailed analysis of serum estrogen and antiestrogen levels was necessary.

The micronized formulation of oral E_2 (Estrace) was chosen for this protocol to facilitate serum monitoring of the biologically active exogenous estrogens. Micronized E_2 is rapidly absorbed into and cleared from the systemic circulation; it is also extensively and variably metabolized to another active estrogen, estrone (E_1) (17). Figures 2 and 3 show the cyclic serum concentrations of E_2 and E_1, as well as TAM and *N*-desTAM, that were achieved during chronic treatment of five patients. These five patients ranged in age from 33 to 56 years and had received from 1 to 19 months of protocol treatment before being sampled. Serum samples were obtained at different times during either antiestrogen (Days 0–27) or estrogen priming (Days 28–56) treatment intervals. The data in figure 2 show that all these patients had baseline postmenopausal E_2 and E_1 levels while receiving TAM (mean $E_2 = 25 \pm 8$; mean $E_1 = 26 \pm 8$ pg/ml). These mean values increased fivefold and tenfold, respectively, during the priming interval (mean $E_2 = 117 \pm 34$; mean $E_1 = 290 \pm 210$ pg/ml) to values approximating those of normal luteal-phase premenopausal women. Both E_2 and E_1 concentrations returned to baseline postmenopausal values at the time TAM therapy was reinitiated, consistent with their known serum half-lives of ≤ 24 hours (17). In contrast to micronized E_2, TAM is known to achieve steady-state serum levels only after days or weeks of administration, and its blood and tissue clearance is similarly delayed (20). Figure 3 shows the cyclical pattern of TAM and *N*-desTAM serum concentrations achieved in the same five patients and over the same treatment cycles as described for figure 2. These data confirm that *N*-desTAM is a major serum metabolite whose concentration exceeds that of the parent compound, TAM (20–22). The wide variations for individual time points are also consistent with previous reports (22). The observed temporal changes were as expected, with both TAM and *N*-desTAM concentrations rising during antiestrogen therapy and falling during estrogen priming. For convenience and according to the known pharmacokinetics of these antiestrogens, mean serum values were calculated for data sets grouped during the early or later phases of treatment with either TAM or E_2 priming. During antiestrogen therapy, the mean TAM serum level rose from 185 (± 76) to 331 (± 122) ng/ml, with mean *N*-desTAM increasing from 207 (± 120) to 375 (± 124) ng/ml. During the early phase of E_2 priming, TAM levels fell 60% to a mean of 134 (± 64) ng/ml, while *N*-desTAM decreased 30% to 258 (± 145) ng/ml. By the later phase of E_2 priming, mean TAM concentration was 40% of its peak level, and *N*-desTAM had declined to 45% of its peak concentration, both values now approximating the mean levels measured during the early phase of antiestrogen treatment. Thus, despite the fact that mean TAM and *N*-desTAM levels persisted > 100 ng/ml (0.3 μM) throughout the entire endocrine treatment cycle, these serum concentrations actually fluctuated over twofold and in opposite phase with the fivefold to tenfold changes measured in E_2 and E_1 levels.

TABLE 3.—Serum levels of TAM and metabolites (*N*-desTAM, 4-OHTAM) in 2 patients during the first endocrine cycle

Patient No.	Time of cycle*	Serum level (ng/ml) TAM	*N*-desTAM	4-OHTAM
1	1A, Day 26	289	433	90
	1B, Day 7	79	149	54
2	1A, Day 21	277	388	< 8
	1B, Day 7	198	417	< 8

*With reference to schematic, fig 1.

At the time of this evaluation, all protocol patients had received an average of 25 weeks of treatment (range, 4–88). Eighteen of these patients had received > 8 weeks of treatment and were evaluated for CR. Five patients (28%) experienced CRs (including three equivocal CRs); two (11%) achieved PRs, and seven (39%) achieved disease stabilization. These patient numbers are too small to demonstrate any significant difference in re-

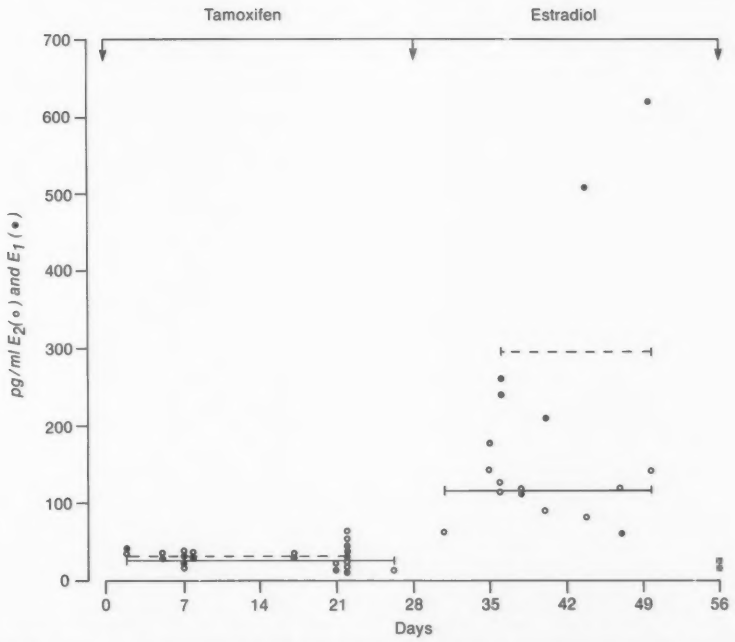


FIGURE 2.—E_2 (○) and E_1 (●) serum levels measured in 5 patients during chronic protocol treatment. As described in fig 1, each endocrine cycle consisted of 2 treatment intervals: TAM therapy (Days 0–27), and estrogen priming in which TAM was discontinued (Days 28–56) and E_2 was administered (Days 28–49). These 5 patients had received from 1 to 19 months of protocol therapy before serum specimens were collected at the times indicated during the sampled treatment interval. Mean E_2 (solid bars) and E_1 (dashed bars) values were calculated from data sets grouped by treatment interval, as shown. During TAM treatment, mean $E_2 = 25 \pm 8$ and $E_1 = 26 \pm 8$ pg/ml. During estrogen priming, mean $E_2 = 117 \pm 34$ and $E_1 = 290 \pm 210$ pg/ml. By the end of this endocrine cycle (Day 56) and with the reinitiation of TAM treatment, E_2 and E_1 levels have returned to baseline postmenopausal values.

sponse rates between the ten patients who had received prior therapy and the eight patients who had not.

DISCUSSION

Preliminary studies utilizing chemoendocrine therapy with estrogen priming have shown encouraging results with CRs > 40% in patients with advanced disease and no prior therapy (3,4,6). Each of these earlier studies employed a 1-hour sequential administration of MTX-FUra, ≤ 10 days of TAM at 20 mg/day, and ≤ 4 days of estrogen priming. One attempt to reproduce these results in heavily pretreated patients failed to achieve any significant responses (5). Several areas of investigation have provided further rationale for improving upon these earlier estrogen priming schedules. These include:

1. Sequencing MTX administration before FUra by 24 hours (rather than 1 hr) further enhances the therapeutic index of this antimetabolite combination (9–11,19).
2. TAM can biochemically interact with MTX-FUra to increase toxicity against ER-positive breast cancer cells (12).
3. TAM requires weeks of administration to achieve steady-state serum levels and a similar duration off treatment for clearance from blood and tissue compartments (20,21).
4. Short-term treatment with estrogen transiently stimulates DNA synthesis of ER-positive tumor cells (13,14); but prolonged estrogen priming may be necessary to sustain enhanced tumor growth in vivo, or to restore growth in quiescent lesions and tumors previously treated with TAM (15).
5. Receptor-binding affinities of TAM and *N*-desTAM are 1/1000 those of E_2 and E_1, indicating that effective cytokinetic modulation by alternating antiestrogen and estrogen therapy must be associated with serum molar ratios of [TAM + *N*-desTAM]/[E_2 + E_1] that fluctuate from > 1000 for growth inhibition to < 1000 for growth stimulation (23–27).

The present chemoendocrine protocol with its prolonged interval of estrogen priming attempts to incorporate each of the above points into a new schedule design. The potential for tumor flare occurring with prolonged E_2 priming necessitated cautious introduction of this pilot study. No tumor flares occurred during any of the > 80 E_2 priming cycles given to these patients. Furthermore, this therapy was well tolerated with most patients experiencing only mild toxicity.

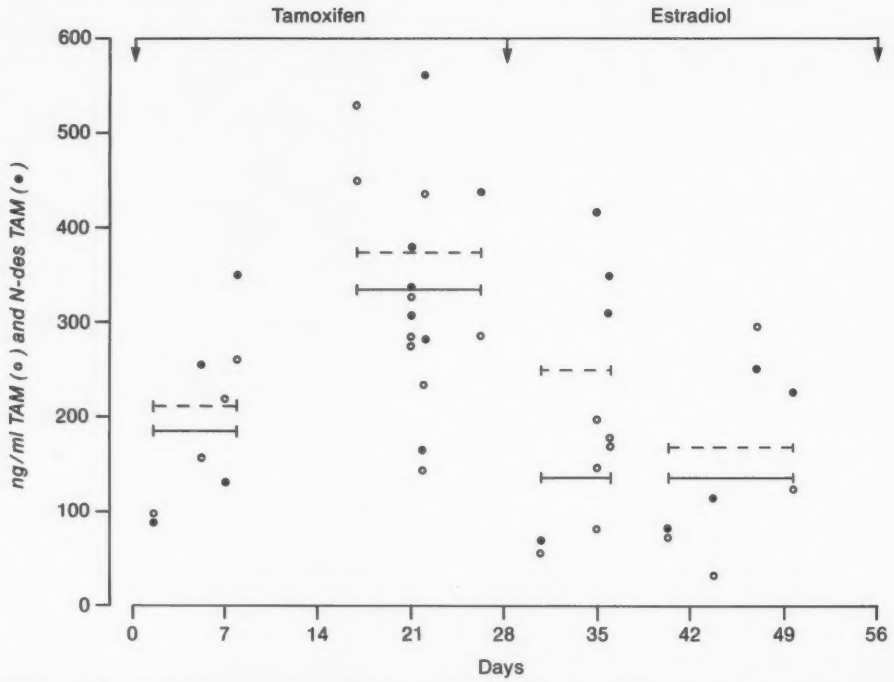


FIGURE 3.—TAM (○) and *N*-desTAM (●) serum levels measured in 5 patients during chronic protocol treatment. Serum specimens were collected at the indicated times during the same treatment intervals and from the same patients as described in fig 2. For convenience, mean TAM (solid bars) and *N*-desTAM (dashed bars) values were calculated from data sets grouped during early or later phases of both TAM and estrogen priming treatment intervals, as shown. Numerical values are provided in the text.

This study is the first to report pharmacologic data associated with the cyclical use of endocrine agents in breast cancer. In our sampled patients, serum levels of TAM and *N*-desTAM rarely declined to < 100 ng/ml, despite the monthly intervals off antiestrogen therapy (during which estrogen priming was administered). In general, steady-state serum levels of 100 ng/ml are routinely achieved with a TAM dose of 20 mg daily and this is usually associated with therapeutic antiestrogen effects (22). Thus, it might be questioned whether estrogen priming, by this or any of the other reported protocols (3-6), is ever actually achieved pharmacologically. Using the mean serum values from figures 2 and 3, however, it is possible to calculate the molar ratios of [TAM + *N*-desTAM]/[E_2 + E_1] for all phases of our endocrine treatment cycle. After the third week of TAM treatment, the mean serum molar ratio is > 10,000, well above the threshold for antiestrogen growth inhibition (> 1000). Following TAM treatment, this ratio is about 700 during the early phase of estrogen priming and then declines to 500. These figures indicate that for most of the estrogen priming interval the molar competition for intracellular ER favors estrogenic growth stimulation, despite persisting serum antiestrogen levels > 100 ng/ml. It is interesting to note that without exogenous E_2 administration, the molar ratios throughout this 4-week interval off TAM would be > 5000, favoring continued antiestrogen effects. Thus, by pharmacologic criteria, estrogen priming is achieved using this protocol schedule.

We did not attempt to assess the resulting effect of cyclic estrogen priming on tumor cell proliferative activity in vivo, although this is of great current interest to us as well as to many other investigators. It is not known whether repetitive cycling of estrogen, given over long or short treatment intervals, is associated with the desired in vivo modulation of tumor cytokinetic parameters. Conte et al (16) have shown that short courses of diethylstilbestrol can increase tumor proliferative activity, measured by thymidine labeling and primer-dependent DNA polymerase labeling; and subsequent chemotherapy can reduce this proliferative activity in vivo. Of interest, this apparent recruitment by estrogens of tumor cells into cycle occurs even in the absence of measurable ER in these tumors (13,16). A full cytokinetic assessment showing cell population arrest, synchronization, and the ability to recycle tumor cells into proliferative activity by hormonal priming has not yet been reported, although modern methods of flow cytometry and fine-needle aspiration make these studies technically feasible.

Lastly, it is encouraging to note that CRs are achiev-

able with this nonaggressive and well-tolerated chemoendocrine regimen. Although these clinical results need to be verified in a much larger patient sample, the observed response rate in our pretreated patient population compares favorably with other estrogen priming studies using similar agents (3–6). Moreover, the minimal toxicity associated with this protocol permits considering future modifications including the addition of anthracycline or alkylating agents, as a rationale emerges supporting their use in combination with endocrine therapy (7,8,28).

REFERENCES

1. LYSS AP, and LOEB V, JR. Chemotherapy of advanced breast cancer, a general survey. Cancer 53:778–782, 1984.
2. ROSE C, and MOURIDSEN HT. Combined cytotoxic and endocrine therapy in breast cancer. *In* Hormones and Cancer 2 (Bresciani F, King RJB, Lippman ME, et al, eds). New York, Raven Press, 1984.
3. ALLEGRA JC, WOODCOCK TM, RICHMAN SP, ET AL. A phase II trial of tamoxifen, premarin, methotrexate and 5-fluorouracil in metastatic breast cancer. Breast Cancer Res Treat 2:93–99, 1982.
4. LIPPMAN ME, CASSIDY J, WESLEY M, ET AL. A randomized attempt to increase the efficacy of a cytotoxic chemotherapy in metastatic breast cancer by hormonal synchronization. J Clin Oncol 2:28–36, 1984.
5. BOWMAN D. A phase I evaluation of sequential tamoxifen, premarin, methotrexate and 5-fluorouracil in refractory stage IV breast cancer. Proc ASCO 2:106, 1983.
6. LIPPMAN M, SORACE R, BAGLEY C, ET AL. Effective systemic management of locally advanced breast cancer. Proc ASCO 4:65, 1985.
7. ZWELLING LA, KERRIGAN D, and LIPPMAN ME. Protein-associated intercalator-induced DNA scission is enhanced by estrogen stimulation in human breast cancer cells. Proc Natl Acad Sci USA 80:6182–6186, 1983.
8. HUG V, JOHNSTON D, FINDERS M, ET AL. Use of growth-stimulatory hormones to improve the in vitro therapeutic index of doxorubicin for human breast tumors. Cancer Res 46:147–152, 1986.
9. BENZ C, CHOTI M, NEWCOMER L, ET AL. Thymidine enhancement of methotrexate and 5-fluorouracil toxicity in cultured human colon carcinoma. Cancer Chemother Pharmacol 12:104–110, 1984.
10. BENZ C, TILLIS T, TATTELMAN E, ET AL. Optional scheduling of methotrexate and 5-fluorouracil in human breast cancer. Cancer Res 42:2081–2086, 1982.
11. BENZ C, SILVERBERG M, and CADMAN E. Use of high-dose oral methotrexate sequenced at 24 hours with 5-FU: a clinical toxicity study. Cancer Treat Rep 67:297–299, 1983.
12. BENZ C, CADMAN C, GWIN J, ET AL. Tamoxifen and 5-fluorouracil in breast cancer: cytotoxic synergism in vitro. Cancer Res 43:5298–5303, 1983.
13. DAO TL, SINHA DK, NEMOTO T, ET AL. Effect of estrogen and progesterone on cellular replication of human breast tumors. Cancer Res 42:359–362, 1982.
14. PARIDAENS RJ, DANGUY AJ, LECLERCQ G, ET AL. Effect of castration and 17B-estradiol pulse on cell proliferation in the uterus and the MXT mouse mammary tumor. JNCI 74:1239–1246, 1985.
15. OSBORNE C, HOBBS K, and CLARK GM. Effect of estrogens and antiestrogens on growth of human breast cancer cells in athymic nude mice. Cancer Res 45:584–590, 1985.
16. CONTE PF, FRASCHINE G, ALAMO A, ET AL. Chemotherapy following estrogen-induced expansion of the growth fraction of human breast cancer. Cancer Res 45:5926–5930, 1985.
17. YEN SSC, MARTIN PL, BURNIER AM, ET AL. Circulating estradiol, estrone and gonadotropin levels following the administration of orally active 17B-estradiol in postmenopausal women. J Clin Endocrinol Metab 40:518–521, 1975.
18. WILBUR BJ, BENZ CC, and DEGREGORIO MW. Quantitation of tamoxifen, 4-hydroxytamoxifen and *N*-desmethyltamoxifen in human plasma by high performance liquid chromatography. Anal Lett 18:1915–1924, 1985.
19. BENZ C, DEGREGORIO M, SAKS S, ET AL. Sequential infusions of methotrexate and 5-fluorouracil in advanced cancer: pharmacology, toxicity, and response. Cancer Res 45:3354–3358, 1985.
20. FABIAN C, STERNSON L, and BARNETT M. Clinical pharmacology of tamoxifen in patients with breast cancer: comparison of traditional and loading dose schedules. Cancer Treat Rep 64:765–773, 1980.
21. JORDAN VC, BAIN RR, BROWN RR, ET AL. Determination and pharmacology of a new hydroxylated metabolite of tamoxifen observed in patient sera during therapy for advanced breast cancer. Cancer Res 43:1446–1450, 1983.
22. TORMEY DC, and JORDAN VC. Long-term tamoxifen adjuvant therapy in node-positive breast cancer: a metabolic and pilot clinical study. Breast Cancer Res Treat 4:297–302, 1984.
23. DARBRE PD, CURTIS S, and KING RJB. Effects of estradiol and tamoxifen on human breast cancer cells in serum-free culture. Cancer Res 44:2790–2793, 1984.
24. JOZAN S, KREITMANN B, and BAYARD F. Different effects of oestradiol, oestriol, oestetrol, and of oestrone in human breast cancer cells (MCF-7) in long term tissue culture. Acta Endocrinol 98:73–80, 1981.
25. MACINDOE JH, WOODS GR, and ETRE LA. The specific binding of estradiol and estrone and the subsequent distribution of estrogen-receptor complexes within MCF-7 human breast cancer cells. Steroids 39:245–258, 1982.
26. HANSEN ER, and BROOKS SC. Estrone receptor formation during the processing of estradiol-receptor complex in MCF-7 cells. Cancer Res 42:1967–1974, 1982.
27. WAKELING AE, and SLATER SR. Estrogen-receptor binding and biologic activity of tamoxifen and its metabolites. Cancer Treat Rep 64:741–744, 1980.
28. SLUYSER M, DEGOEIJ CCJ, and EVERS SG. Combined endocrine therapy and chemotherapy of mouse mammary tumors. Eur J Cancer 17:155–159, 1981.

Small Cell Carcinoma of the Lung: Influence of Age on Treatment Outcome[1,2]

Elizabeth Poplin,* Bruce Thompson, Margaret Whitacre, and Joseph Aisner[3]

Two hundred twenty-three patients between 29 and 78 years of age with small cell cancer of the lung were studied in serial protocols at the University of Maryland Cancer Center from 1975 to 1982. Each of these patients received chemotherapy consisting of doxorubicin, cyclophosphamide, and etoposide. In this paper we explore age and its interaction with the patient's extent of disease as it relates to clinical outcome variables such as the achievement of a complete response (CR), survival times, and various measures of toxicity. Preliminary evidence is found to suggest that an age-extent of disease interaction affects both the probability of obtaining a CR and the length of survival. Patients of all ages can benefit from treatment of small cell carcinoma of the lung, with CR as the treatment goal. However, increased toxicity and early death remain as barriers to successful treatment of the elderly. [Cancer Treat Rep 71:291-296, 1987]

Recent advances in the treatment of small cell carcinoma of the lung (SCCL), mandating the initial use of aggressive chemotherapy with or without radiotherapy, have dramatically improved the prognosis of this disease. Previously, the natural history of this disease was that of rapid progression, with a median survival < 3 months and virtually no patient alive at 1 year (1). Currently, an appropriately treated patient with SCCL has a high likelihood of response to therapy, with significant prolongation of survival (2-10). Among the patients presenting with locoregional disease, aggressive therapy has been shown to achieve a 60% complete response rate and a 20%-30% long-term disease-free survival rate, with some patients alive at 8 years' follow-up (8,11-16).

SCCL is predominantly a disease of the middle aged to older smoking population. The median age at diagnosis is approximately 60 years. However, the diagnosis is made in patients ≥ 80 years of age. Despite the known potential survival advantage with treatment, many physicians caring for older patients may be reluctant to recommend aggressive therapy fearing the toxic effects of chemotherapy or combined modality treatment. At the University of Maryland Cancer Center (UMCC), we have been treating patients of all ages with SCCL with a common treatment strategy of aggressive chemotherapy (18). The purpose of this report is to examine response frequencies, survival times, and toxic effects among various age groups receiving the same therapy for SCCL and specifically to clarify the benefits and hazards of this therapy for the older patient.

MATERIALS AND METHODS

For the purpose of this review, charts of all patients treated with ACE chemotherapy (doxorubicin, cyclophosphamide, and etoposide) in the protocol (table 1) or with the same three drugs but not in the study because of protocol exclusion criteria were reviewed to assess response, duration of survival, and toxicity. There were no age restrictions for either the three sequential protocols or the patients who were excluded because of protocol ineligibility.

Data were grouped by patient age at diagnosis: < 55 years; 55-59 years; 60-64 years; and ≥ 65 years. The sex of the patient was also assessed and included in the data base. Performance status (PS) was assigned according to Cancer and Leukemia Group B (CALGB) criteria at the time of initiation of treatment. Limited disease (LD) was defined as disease confined to one hemithorax with or without bilateral hilar, mediastinal, or ipsilateral supraclavicular lymph node involvement. Patients with disease more widespread than that noted above were considered to have extensive disease (ED).

All efforts were made to give full-dose chemotherapy without delay. First-course doxorubicin dose reduction was stipulated in the first two protocols for patients with hepatic function compromise (bilirubin elevation). Otherwise, all patients received a full dose initially, with subsequent reductions in dose for myelosuppression. Chest irradiation was not used in these protocols. Prophylactic cranial irradiation (PCI), given after con-

[1] Received Feb 10, 1986; revised Nov 25, 1986; accepted Dec 5, 1986.
[2] Supported in part by Public Health Service grant CA-32107 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] Division of Medical Oncology, Department of Medicine, University of Maryland Cancer Center, Baltimore.
* *Reprint requests to:* Elizabeth A. Poplin, MD, Wayne State University, PO Box 2188, Detroit, MI 48201.

TABLE 1.—Summary of patients receiving protocols*

Protocol No.	Regimen	PCI	Dates
7510	ACE ± MER	No PCI	6/75–7/77
7705	ACE alternating with ACE/COMP	PCI in CR (randomized)	8/77–9/80
8020	ACE vs ACE-II	PCI in CR	10/80–1/83

*A = doxorubicin (45 mg/m^2 iv on Day 1); C = cyclophosphamide (1000 mg/m^2 iv on Day 1); E = etoposide (50 mg/m^2 on Days 1–5); MER = methanol-extracted residue of BCG (200 μg) in 5 sites intradermally; C = lomustine (75 mg/m^2 iv on Day 1); O = vincristine (1 mg/m^2 iv on Days 1 and 21); M = methotrexate (40 mg/m^2 iv on Days 1 and 21); P = procarbazine (75 mg/m^2 orally on Days 1–5 and 21–25); and ACE-II = ACE with etoposide (100 mg/m^2/day by continuous infusion on Days 1–5). Each cycle for all 3 regimens = 21 days.

firmation of complete response (CR), was adopted during the second study after a demonstration of its value (17). Patients continued treatment with ACE in the absence of progressive disease for 24 months.

Responses were assessed at the time of maximal clinical response or after the third treatment course or both. Standard criteria of response were used (4). Restaging included repeat chest radiographs, chest tomography, or computerized tomographic scan of chest; at a minimum, all initial abnormal studies were repeated. Because of the use of dose modifications, hematologic toxicity was evaluated using a first-course wbc and platelet count nadir. Other toxicity tabulated included admissions for infection or febrile illness on initial courses and admissions during subsequent treatment for infection or febrile illness while granulocytopenic. The above variables formed the basis of our studies.

Statistical Methods

Dependent variables were modeled using the Cox proportional hazards model for time to event data, logistic regression for binary data, and linear regression for continuous data which were not time-oriented (19,20). In all patients all of the above covariables were included in the model while assessing the significance of any other covariable. Thus, stepped procedures as commonly used in these types of studies were not utilized in our analysis. Should a particular variable or interaction prove to be significant, conventional techniques such as Fisher's exact test and t tests were used to highlight these observations.

PATIENT CHARACTERISTICS

Two hundred seventeen patients were treated with ACE in the protocol. An additional six patients with previously treated malignancies in control at the time of treatment initiation received the same treatment and are included for evaluation of age-dependent effects. Fifty-six patients in Study 7705 received ACE alternating with COMP (lomustine, vincristine, methotrexate, and procarbazine), after a minimum of three courses of ACE induction. Patients relapsing or progressing on ACE were treated with either the COMP combination,

TABLE 2.—Classification of patients in each group

	Age group (yrs)				
	≤ 54	55–59	60–64	≥ 65	Total
No. of patients with LD	17	21	30	24	92
PS*	1 (0.45)	1 (0)	1.5 (1)	2 (2)	
No. of patients with ED	34	30	32	25	121
PS*	2 (2)	1 (1.5)	2 (2)	2 (2)	
Total	51	51	62	49	213

*Values = median PS (interquartile range).

radiation therapy to sites of disease, or new phase II agents according to ongoing protocols and patient needs. Ten patients are excluded from analysis of response and survival because of unrecorded responses (three patients), concurrent malignancies (three), or loss to follow-up (four). Twelve additional patients are excluded from Cox hazard and logistic regression analysis because PS was not documented. The survival times of three additional patients who developed secondary malignancies are censored at the time of the second tumor diagnosis. Patient age ranged from 29 to 78 years. Table 2 shows a cross-classification of extent of disease and age group and median PS for each group. A plurality of patients were 60–64 years of age. One hundred twenty-one patients had ED. Males outnumbered females 2.5:1. Neither initial PS nor baseline wbc and platelet counts varied significantly between groups.

RESPONSE

Multiple logistic regression analysis indicated that CR frequency varied significantly according to the age and extent of disease status of the patient ($P = 0.052$) (table 3). On closer inspection of table 3, it can be seen that the major components of this interaction are a smaller response rate among the young patients with LD and a higher response rate among the younger patients with ED. Of the other independent covariables analyzed (PS and sex), none were found to be significantly associated with response.

TABLE 3.—Response rates for 213 patients classified by age and extent of disease

Extent of disease	Age group (yrs)			
	≤ 54	55–59	60–64	≥ 65
LD				
CR proportion*	0.41 ± 0.12	0.71 ± 0.099	0.63 ± 0.089	0.75 ± 0.088
Total No. of patients	17	21	30	24
ED				
CR proportion*	0.59 ± 0.084	0.37 ± 0.088	0.34 ± 0.084	0.4 ± 0.098
Total No. of patients	34	30	32	25

*± SE.

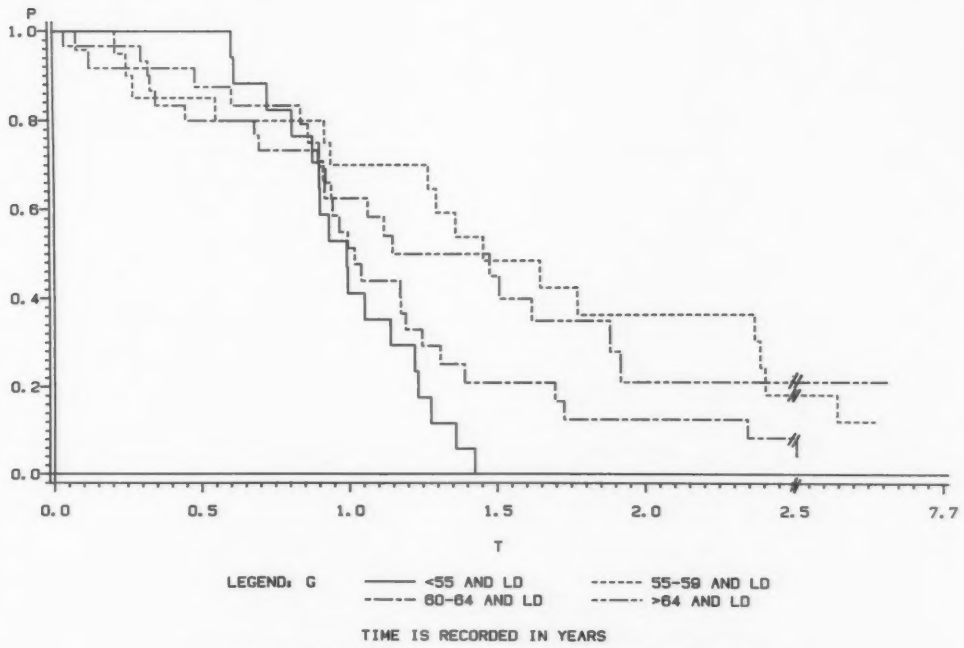


FIGURE 1.—Kaplan-Meier survival plots for different ages; all patients had LD.

Survival

Cox proportional hazards analysis using age, extent of disease, PS, sex, and an age-extent of disease (ED) interaction between independent variables revealed that only the PS ($P = 0.0001$) and interaction between a patient's age and extent of disease ($P = 0.04$) were significantly associated with survival time. For a visual comparison, figures 1 and 2 show the Kaplan-Meier survival curves for each age-extent of disease category. It is interesting to note that the direction of this interaction is just what would be expected, according to the type of interaction observed in the response analysis. That is to say, the age-extent of disease groups which yielded the highest response rates also exhibit the longest survival periods.

Toxicity

Significant toxicity including myelosuppression, neutropenic febrile episodes, and numbers of hospitalizations for infection or febrile illnesses associated with neutropenia were reviewed, and summary figures are given in table 4. Both the mean first-course wbc and platelet count nadirs varied significantly with age ($P = 0.0003$ and 0.013, respectively). Closer examination of the means for these two toxic effects using an analysis of variance showed the following. (*a*) For platelet count nadir, there is a directional response with age; there is a more profound thrombocytopenia with increasing age. (*b*) There is a more profound thrombocytopenia with ED and CR. (*c*) There is greater platelet suppression with CR. A similar analysis for wbc count nadirs showed a directional relationship for several factors, ie, there was more profound leukopenia with increasing age, ED, and male sex. Thus, the consistent relationships for myelosuppression were ED and increasing age. The age-extent of disease interaction was of borderline significance ($P = 0.06$) for both thrombocytopenia and leukopenia.

The severity of myelosuppression in many patients was associated with occurrence of febrile episodes. First-course admissions for neutropenia-associated febrile illnesses occurred in 78 of 207 evaluable patients (38%). Admission for such episodes occurred with increased frequency in the two older age groups, 60–64 and 65 years, with 42% and 47%, respectively, requiring admission compared to those among younger groups, with only 34% of those ages 54 years and 27% of those ages 55–59 years requiring admission. Both poorer initial PS ($P = 0.0001$) and greater extent of disease ($P = 0.03$) were predictive of need for first-course admission for toxicity.

Despite dose modifications, admissions for febrile illnesses while receiving ACE chemotherapy were frequent in subsequent courses as well, with 68 of 192 patients requiring admission. Admissions were required in 29%, 37%, 33%, and 44% of patients in the four sequential age groups.

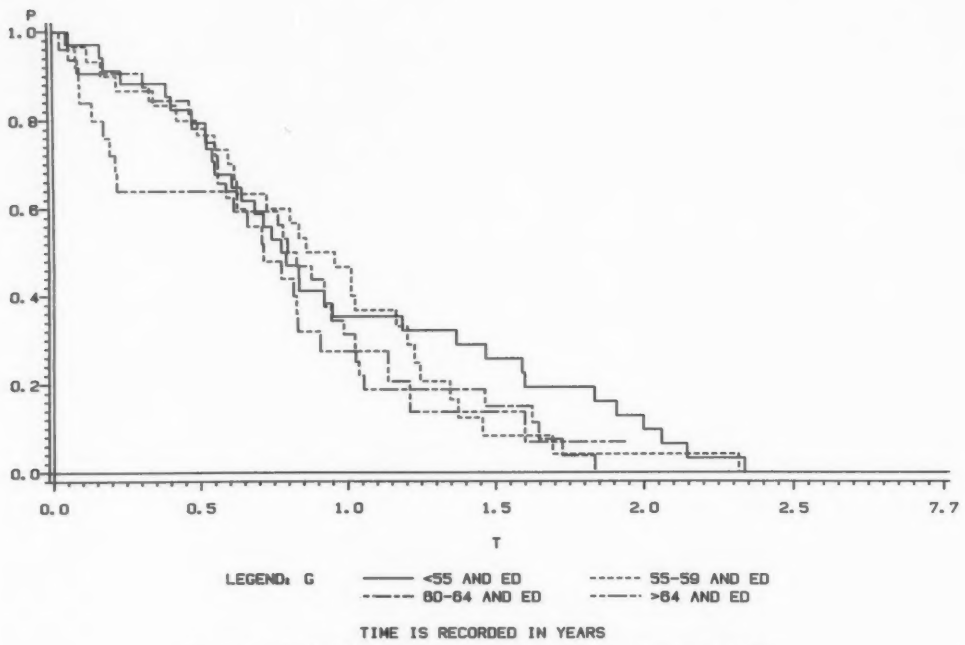


FIGURE 2.—Kaplan-Meier survival plots for different ages; all patients had ED.

Cardiotoxicity

CHF was seen and required termination of therapy in eight patients. The youngest patient, 57 years old, had had emergency radiotherapy to the mediastinum before receiving chemotherapy. Four other patients, 60–64 years of age, also developed signs of CHF. Among patients 70 years of age, three were noted to develop CHF after 2, 3, and 16 months of treatment.

OUTCOME

Of 223 patients, six are excluded because of concurrent or subsequent development of malignancies. Twenty-one patients remain alive, and 151 have died of progressive disease. Cause of death could not be ascertained in 14 other patients including four lost to follow-up. One patient died from *Legionella* pneumonia and a second from progressive chronic respiratory failure with stable disease.

TABLE 4.—Patient characteristics and toxicity*

Characteristic	Age group (yrs)			
	≤ 54	55–59	60–64	≥ 65
Nadir wbc count ($\times 10^3/mm^3$)	1.02 (48)	0.75 (48)	0.77 (56)	0.33 (48)
Nadir platelet count ($\times 10^3/mm^3$)	156 (48)	155 (47)	150 (54)	103 (48)
First-course neutropenic admissions	34% (51)	27% (51)	42% (62)	47% (49)
Multiple febrile admissions	29% (51)	37% (51)	33% (62)	44% (49)
No. of deaths†	5 (51)	7 (51)	7 (62)	10 (49)

*Values in parentheses = No. of patients.
†Includes sudden deaths and deaths from toxic effects.

Sudden Deaths

Of the remaining 29 patients, ten deaths were sudden and 19 toxic. Of the sudden deaths, two resulted from myocardial infarction, one from an apparent pulmonary embolus, and a fourth from an exsanguinating bleed in the absence of thrombocytopenia. Three additional patients had granulocytopenia at the time of their deaths at home. Cause of sudden death in the remaining three patients was not obvious. Of the toxic deaths, sepsis contributed significantly to the deaths of 16 patients. One additional patient died from multiple problems including gastrointestinal bleeding, neurologic deterioration, and respiratory failure. Of the 29 toxic and sudden deaths, five occurred in patients ≤ 54 years of age, seven each among patients 55–59 and 60–64 years of age, and ten among patients ≥ 65 years of age. When death from all causes was evaluated, initial poorer PS ($P = 0.0025$) but not age or extent of disease was significant in predicting sudden/toxic death.

DISCUSSION AND CONCLUSION

The results of chemotherapy and combined modality treatment of SCCL have dramatically altered the short-

and long-term outlook for this disease. Most single-institution studies using aggressive combination chemotherapy demonstrate a > 80% total response rate, a frequency of CR > 60% for LD patients and > 20% for ED patients, and a disease-free survival > 3 years for approximately 20% of patients presenting with LD (2).

Understandably, physicians question whether the overall improvement in outlook with regard to response and survival for patients with SCCL pertains to the older patients with this disease and whether such improvement occurs only at the expense of intolerable toxicity. However, only limited data are available from past studies. A review of Southwest Oncology Group data of patients treated with combined modality treatment showed a statistically significant higher frequency of CR in patients with LD who were < 55 years of age compared to those who were older. In ED patients, no such difference was seen (21). Survival was not analyzed by age. At Memorial Sloan-Kettering Cancer Center, Sierocki et al (22) noted that there was a lower median age among those living > 1 year compared to those living a shorter time. Østerlind et al (23) also noted that age > 60 years was associated with poorer survival. In a CALGB compilation of data derived from multiple protocols comparing various combinations of chemotherapeutic agents with single-agent cyclophosphamide for SCCL, age was also found to be prognostically of some significance (24). Patients > 70 years of age with LD tended to have a lower CR rate than did those who were younger. No difference was noted among ED patients. There was also a trend toward lower median survival among those patients > 70 years of age, while long-term survivors were usually < 60 years of age.

Our data, in contrast to trends suggested by the above studies, find a surprisingly low CR rate among the youngest LD patients, but response rates among older LD and ED patients were fairly stable in successive age groups. No satisfactory explanation of the low response rate in the youngest LD group can be given at this time, and it may prove to be a statistical artifact. However, it is possible that, among the youngest patients, the tumor may indeed be less responsive and that this behavior is more easily demonstrated in patients with minimal disease. Analysis of many more patients in this age population by us and others will be necessary to confirm and extend this observation.

Survival outcome was primarily affected by initial PS, as might be expected (25). Interestingly, we found that an extent of disease-age interaction was also significantly associated with survival. The fact that the pattern of survival coincided with the CR rate suggests that the response advantage experienced by some age-extent of disease groups carries over into increased survival advantages for these patients. We hope that future treatment trials will analyze survival not only according to PS and extent of disease but also by age and the interaction of extent of disease and age to confirm our observation.

Most studies show significant toxicity of either chemotherapy or combined modality, although without characterization of age-related effects. In general, febrile illnesses are seen in about 30% of patients, documented infections in 5%–10%, and lethal toxic effects in about 5% (2,26). In our patients, myelosuppression was severe in all age groups, with first-course myelosuppression being more severe in patients ≥ 65 years of age and first-course admissions for febrile granulocytopenic episodes being more frequent in those ≥ 60 years of age. The frequency of toxic and sudden deaths was not significantly increased among older patients, although a trend ($P = 0.2$, two-sided) was seen. Further analyses in larger series may help to clarify this issue.

Because of the increased severe myelosuppression in the older patient and the need for hospitalization, additional vigilance on the part of the physician will be required. Prophylactic oral antibiotics as used at UMCC (27) may be useful. Prophylactic hospitalization during periods of aplasia, as described by Markman and Abeloff (28), should be considered, especially during early cycles of treatment when increased toxicity, occurring as a result of full-dose therapy and the clinical manifestations of active disease, may be associated with greater risks of fever and infection. Early institution of systemic antibiotics for fever in a granulocytopenic or soon-to-be granulocytopenic host is critically important.

These data suggest that patients of all ages with SCCL should and can be treated aggressively and vigorously for their disease. Responses among the oldest patients appear as attainable and as durable as those among the younger patients. Since all patients, particularly the elderly patients, who do not achieve CR have a poor survival, additional efforts must be directed toward obtaining CR in a greater proportion of all patients.

REFERENCES

1. HYDE L, YEE J, WILSON R, ET AL. Cell type and the natural history of lung cancer. JAMA 193:140, 1965.
2. AISNER J, ALBERTO P, BITRAN J, ET AL. Role of chemotherapy in small cell lung cancer: a consensus report of the International Association for the Study of Lung Cancer workshop. Cancer Treat Rep 67:37-43, 1983.
3. ISSELL B, RUDOLPH A, LAWSON R, ET AL. Cytoxan, etoposide and vincristine versus Cytoxan, Adriamycin and vincristine or Cytoxan and vincristine in the treatment of small cell carcinoma of the lung. Proc ASCO 2:C-748, 1983.
4. AISNER J, and WIERNIK PH. Chemotherapy vs. chemoimmunotherapy for small cell undifferentiated carcinoma of the lung. Cancer 46:2543, 1980.
5. SHANK B, NATALE RB, HILARIS BS, ET AL. Treatment of small cell carcinoma with combined high dose mediastinal irradiation, whole brain prophylaxis and chemotherapy. Int J Radiat Oncol Biol Phys 7:469, 1981.
6. COX JD, HOLOYE PY, BYHARDT RW, ET AL. The role of thoracic and cranial irradiation for small cell carcinoma of the lung. Int J Radiat Oncol Biol Phys 8:191, 1982.
7. HOLOYE PY, SAMUELS ML, LANZOTTI VJ, ET AL. Combination chemotherapy and radiation therapy for small cell carcinoma. JAMA 237:1221, 1977.
8. LYMAN GH, HARTMANN RC, SABA HI, ET AL. Combination chemotherapy and radiation therapy of undifferentiated small cell bronchogenic carcinoma. South Med J 71:519, 1978.

9. DILLMAN RO, TAETLE R, SEASREN S, ET AL. Extensive disease small cell carcinoma. Cancer 49:2003, 1982.
10. ARONEY RS, BELL DR, CHAN WK, ET AL. Alternating non-cross resistant combination chemotherapy for small cell anaplastic carcinoma of the lung. Cancer 49:2449, 1982.
11. SCHER H, HILARIS B, and WITTES R. Long term followup of the combined modality therapy in small cell carcinoma of the lung. Proc ASCO 2:C-777, 1983.
12. HANSEN M, HANSEN HH, and DOMBERNOWSKY P. Long term survival in small cell carcinoma of the lung. JAMA 244:247, 1980.
13. VAN HOUTTE P, TANCINI G, DE JASER R, ET AL. Small cell carcinoma of the lung: a combined modality treatment. Eur J Cancer 15:1159-1165, 1979.
14. EINHORN LH, BOND WH, HORNBACK N, ET AL. Long term results in combined modality treatment of small cell carcinoma of the lung. Semin Oncol 5:309, 1978.
15. GINSBERG SJ, COMIS RL, GOTTLIEB AJ, ET AL. Long-term survivorship in small-cell anaplastic lung carcinoma. Cancer Treat Rep 63:1347-1349, 1979.
16. OLDHAM RK, and GRECO FA. Small cell lung cancer. Cancer Chemother Pharmacol 4:173, 1980.
17. AISNER J, WHITACRE M, VAN ECHO DA, ET AL. Combination chemotherapy for small cell carcinoma of the lung: continuous vs. alternating non-cross-resistant combinations. Cancer Treat Rep 66: 221-230, 1982.
18. AISNER J, WHITACRE M, VAN ECHO DA, ET AL. Doxorubicin, cyclophosphamide and VP16-213 (ACE) in the treatment of small cell lung cancer. Cancer Chemother Pharmacol 7:187, 1982.
19. ARMITAGE P, MCPHERSON CK, and COPAS JC. Statistical studies of prognosis in advanced breast cancer. J Chronic Dis 22:343-360, 1969.
20. COX DR. Regression models and life tables. J Stat Soc 34:187-220, 1972.
21. REED RC, LIVINGSTON RB, and SOUTH WEST ONCOLOGY GROUP. Prognostic significance of pretreatment variables in small cell carcinoma of the lung. Proc Am Assoc Cancer Res and ASCO 18:307, 1977.
22. SIEROCKI JS, HILARIS BS, HOPFAN S, ET AL. Small cell carcinoma of the lung. Experience with six-drug regimen. Cancer 45:417-422, 1980.
23. ØSTERLIND K, IHDE DC, ETTINGER DS, ET AL. Staging and prognostic factors in small cell carcinoma of the lung. Cancer Treat Rep 67:3-9, 1983.
24. MAURER LH, and PAJAK TF. Prognostic factors in small cell carcinoma of the lung: a Cancer and Leukemia Group B study. Cancer Treat Rep 65:767-774, 1981.
25. ZELEN M. Keynote address on biostatistics and data retrieval. Cancer Chemother Rep Part 3, vol 4(2):31-42, 1973.
26. ABELOFF MD, KLASTERSKY J, DRINGS PD, ET AL. Complication of treatment of small cell carcinoma of the lung. Cancer Treat Rep 67:21-26, 1983.
27. DEJONGH CA, WADE JC, FINLEY RD, ET AL. Trimethoprim/sulfamethoxazole versus placebo: a double-blind comparison of infection prophylaxis in patients with small cell carcinoma of the lung. J Clin Oncol 1:302-307, 1983.
28. MARKMAN M, and ABELOFF M. Management of the hematologic and infectious complication of intensive induction therapy for small cell carcinoma of the lung. Am J Med 74:741-746, 1983.

Use of Nude Mouse Xenografts as Preclinical Drug Screens: In Vivo Activity of Established Chemotherapeutic Agents Against Melanoma and Ovarian Carcinoma Xenografts[1,2]

Raymond Taetle,[3,*] Fred Rosen,[3] Ian Abramson,[3] John Venditti,[4] and Stephen Howell[3]

To evaluate the utility of nude mouse xenografts as preclinical drug screens, the activity of ten established chemotherapeutic agents was evaluated against seven melanoma and three ovarian carcinoma xenografts. Xenografts were established using primary explants from patients who had not received chemotherapy and serially passaged as sc tumors in nude mice. In vivo drug activities for dactinomycin, carmustine, vinblastine, melphalan, amsacrine, cisplatin, bleomycin, mitomycin, doxorubicin, and etoposide were evaluated by 4 weekly ip injections of 10% less than LD10 doses. Plots of relative tumor growth versus time were nearly log-linear. Analysis of in vivo activity was performed using percent control growth (treated/control tumor volume) and by calculation of a novel growth delay index obtained by fitting growth curves to a quadratic regression model. Both modes of data analysis identified alkylating agents (melphalan, carmustine, and mitomycin) as the most active drugs against human melanomas. Melphalan, mitomycin, and cisplatin showed the greatest activity against ovarian xenografts. However, complete tumor regressions were noted only with melphalan, mitomycin, and cisplatin against a single ovarian tumor xenograft. Correlation analysis suggested xenograft tumor growth rate was an important determinant of drug response. These results suggest that preclinical, new-drug screening with melanoma xenografts would identify drugs such as alkylating agents as active, and may not provide an advantage over murine leukemia screens. However, screening with ovarian xenografts may more closely reflect clinical drug activity. Criteria for detecting active drugs in such systems are discussed. [Cancer Treat Rep 71:297-304, 1987]

Human tumors transplanted into athymic (nude) mice represent a model for examining human solid tumor responses to new and established chemotherapeutic agents (1-5). Although new agents have been tested against xenografts to assess preclinical activity, the types of drugs identified as active by such systems remain unclear (6). As part of a study comparing in vivo and in vitro drug responses by xenografts (7), we examined in vivo responses by melanoma and ovarian carcinoma xenografts to ten established chemotherapeutic agents. To facilitate comparisons between drugs, a novel semiquantitative index was developed to measure in vivo drug effects.

MATERIALS AND METHODS

Athymic Mice

Nude mice, originally of BALB/c background, 6-8 weeks of age, were obtained on a weekly basis from the Frederick Cancer Research Facility, National Cancer Institute, MD. The animals were housed in a vivarium equipped with HEPA filters and controlled for temperature (76°F-80°F), humidity (50%-55%), and light cycle (12 hours light and dark). The animals were provided with sterilized food and water ad libitum and kept in groups of five in plastic cages fitted with polyester fiber filter covers. Clean, sterilized gowns, gloves, face masks,

[1] Received Apr 9, 1986; revised Nov 18, 1986; accepted Dec 5, 1986.

[2] Supported in part by contract N01-CM-37568 (Division of Cancer Treatment) and Public Health Service grant CA-23100 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services; by a grant from the Cancer Research Coordinating Committee, University of California; and by the Clayton Foundation for Research, California Division. S. Howell is a Clayton Foundation investigator.

[3] Departments of Medicine (*R. Taetle, F. Rosen,* and *S. Howell*), Pathology (*R. Taetle*), and Mathematics, and Cancer Center (*I. Abramson*), University of California, San Diego.

[4] Drug Evaluation Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

* *Reprint requests to:* Raymond Taetle, MD, University of California, H720, 225 Dickinson St, San Diego, CA 92103.

and shoe and head covers were used by all personnel handling the animals.

Xenograft Tumor Establishment and Transplantation

Twelve metastatic human melanomas and 11 ovarian tumors were obtained from patients who had not received chemotherapeutic agents. Two xenografts (melanomas 354 and 355) were established from different explants from the same patient. Samples (0.5–5.0 g) of freshly excised tumor were placed in Dulbecco's minimum essential medium (MEM) (Grand Island Biological Co, NY) containing 1% penicillin/streptomycin (Gibco), 5% fetal bovine serum (Microbiological Associates, Rockville, MD), and 10% horse serum (Irvine Scientific, Santa Ana, CA). Under sterile conditions the tumor was trimmed of necrotic tissue and a small piece placed in buffered Formalin for histology. Following removal of the encapsulating membrane, the tumor was minced and suspended in cold MEM containing antibiotics (5 ml/g of tissue). After centrifugation at low speed for 5 minutes, the supernatant was discarded and the tumor mince transferred to a 3-ml syringe. The tissue was gently pressed through a 16-gauge needle into another syringe, and 0.4 ml of the suspension was implanted sc into the right flank of multiple animals. Ovarian xenografts were implanted and passaged only in female mice. The time from tumor acquisition to implantation was usually < 2 hours. For experimental studies, the tumors were prepared as above, and 0.2 ml of the suspension was transplanted per animal.

The xenografts studied were as follows (passage numbers shown in parentheses): melanomas 242 (44th, 53rd); 354 (30th, 32nd); 355 (6th, 7th); 508 (4th, 5th); 521 (3rd, 5th); 539 (1st, 3rd); 289 (5th, 7th), and ovarian carcinomas 17 (30th, 31st); 428 (24th, 26th); and 513 (6th, 7th).

For the first five passages, then subsequently on every third passage, the tumors were assayed for LDH isoenzymes as described previously (8). An arbitrary limit of 5% was fixed as the maximum amount of mouse LDH isoenzyme allowed in the human xenografts. At various passages, xenograft samples were frozen and stored in liquid nitrogen. The original tumor and selected passages were histologically examined to confirm the original diagnosis (performed by Dr. Virginia Swanson, University of California, San Diego). Seven melanoma and three ovarian carcinomas were expanded into sufficient numbers of mice for in vivo drug testing.

Drugs

Drugs were obtained from the Pharmaceutical Resources Branch, Division of Cancer Treatment, National Cancer Institute. The drugs studied were: dactinomycin, doxorubicin, amsacrine (*m*-AMSA), carmustine (BCNU), bleomycin, cisplatin, melphalan, mitomycin, vinblastine, and etoposide.

Appropriate aliquots of each batch of drug were partitioned into 50 vials. Five aliquots were removed and assayed spectrophotometrically for drug concentration. The average coefficient of variation for drug concentrations in the prepared vials was 6.6% (range, 1.7%–15.6%). The capped vials were stored in a refrigerator at 4°C and were diluted just prior to use. BCNU was dissolved in absolute alcohol and then diluted with water. Melphalan was dissolved in 0.1 ml of acid/alcohol and 0.9 ml of phosphate buffer in propylene glycol (pH 7.0) followed by normal saline. *m*-AMSA was dissolved in 0.25 ml of *N,N*-dimethylacetamide, followed by 0.035 M lactic acid and water. All other drugs were dissolved in normal saline. Drugs were administered once a week using a fresh vial of drug. Because these studies were part of a larger project comparing in vivo and in vitro drug effects, agents requiring metabolic activation, such as dacarbazine, were not included.

LD10 Determination

For each drug, the LD10 in BALB/c nude mice bred in our colony was determined using the following protocol. Each dose of drug was given to five male and five female mice 6–8 weeks of age weighing from 16 to 25 g. The drugs were freshly prepared on the day of injection and injected ip weekly for 4 weeks. Animals were observed for an additional 4 weeks. The animals were observed daily and weighed once a week during this 8-week period. Using probit log paper, percent dead animals was plotted against log drug dose. Regression lines were calculated using least-squares analysis and the LD10 estimated from the regression line. The LD10 doses (mg/kg) were: dactinomycin, 0.08; doxorubicin, 2.9; *m*-AMSA, 2.8; BCNU, 29.6; bleomycin, 25.8; cisplatin, 3.5; melphalan, 10.5; mitomycin, 2.7; vinblastine, 0.84; and etoposide, 4.3.

Chemotherapy

Each treatment group as well as the untreated control group consisted of nine to ten mice. Animals of the same sex, 6–8 weeks of age and weighing 17–25 g, were used in replicate or triplicate experiments. Each experiment consisted of 11 groups of animals per tumor xenograft, ten treated with different drugs and one control group. When tumors reached an average size of 0.5–1.0 cm in diameter (2–5 weeks), tumor-bearing animals were randomized by a card system. Five animals were housed in each cage and identified by an ear punch. All drugs were administered ip at a dose 10% less than the LD10 every 7th day for a total of four treatments. Drug treatment, tumor diameter, and animal weight measurements were performed on the day the animals were randomized. Thereafter, tumor diameters and weights were recorded once a week for 4 weeks. The animals were terminated beginning 2–9 weeks after the last drug injection.

Measurement of Tumor Weight

Starting on the first day of treatment and weekly thereafter, tumor size was followed by caliper measure-

ment of two perpendicular diameters. Tumor weight (W) in mg was estimated by the method of Geran et al (9) using the formula: $W = (\text{width, mm})^2 \times (\text{length, mm})/2$. Tumor measurements for each xenograft were converted from absolute to relative values. The relative mean tumor weight (RW) was calculated from the formula: $RW = W_i/W_o$, where W_i is the mean tumor weight for the group of animals at any given time and W_o is the mean tumor weight just prior to initial treatment.

Statistical Evaluation

Data were entered into a Sun computer using a UNIX operating system and MUMPS file manager. To analyze drug effects over the entire course of experiments, tumor growth was postulated to follow a parabolic curve of the form:

$$y = \alpha_{control}\, t + \beta_{control}\, t^2 + \text{error},$$

where y is the log of the ratio of tumor weight at time t to the initial tumor weight, and the errors are assumed to be independent, centered normal disturbances. Data for drug-treated groups were fitted to the identical equation. These represent linear models for the α and β coefficients which can be estimated by standard regression analysis.

Because the duration of experiments was fixed at 4 weeks, a measure of tumor growth delay can be derived from the integrated difference between the control and drug-treated curves fitted to the equations shown above. A growth delay index (GDI) was calculated using the formula:

$$GDI = \frac{(\alpha_t - \alpha_c)}{2}\, t^2max + \frac{(\beta_t - \beta_c)}{3}\, t^3max,$$

where t_{max} is the duration of the experiment (maximum time, 4 weeks). The number derived (GDI) represents a difference between the two curves in arbitrary units. The procedures for evaluating growth curves will be discussed in detail elsewhere.[5]

Rates of animal deaths and animal weights between groups were compared using a one-way analysis of variance. Correlations between tumor growth rate and tumor regression were performed using the standard product moment formula. These were performed for the aggregate data base using all drug-tumor pairs and for each drug separately. Relationships between tumor passage number and drug effects were examined using a two-way analysis of variance.

RESULTS

Growth of Tumors in Nude Mice

Melanomas 242, 354, and 355 and ovarian tumors T17 and T428 were established several years ago (10) and liquid-nitrogen-frozen samples were used to re-establish these tumors. Over an 18-month period, five of 12 (42%) melanomas and five of 11 (45%) ovarian tumors were implanted into nude mice, showed growth beyond the second passage, and were considered established xenografts. Five of seven primary ovarian carcinoma implants, which initially grew slowly, showed a marked increase in growth rate when mice were treated sc with one unit of follicle-stimulating hormone three times a week. However, follicle-stimulating hormone-dependent tumors were not used in drug trials. The mean time required from tumor implantation to first passage was 16 weeks (range, 8-36). Times between subsequent passages for a tumor varied over a period of 3-8 weeks, showing a characteristic pattern for each tumor. Rates of tumor growth could be enhanced by doubling the transplanted volume of tumor mince from 0.2 to 0.4 ml.

Drug Toxicity

Death rates during drug treatment studies ranged from 5% to 28% and were not significantly different for control and drug-treated groups. Thus, although individual tumor growth data were censored for animal deaths, this occurred with equal frequency among control and treated animals and should not alter evaluation of drug effects. Average animal weights were also examined and did not differ significantly between control and drug-tested groups.

Xenograft Responses to Established Chemotherapeutic Agents

Figures 1 and 2 show representative growth curves obtained using two melanoma and two ovarian carcinoma xenograft lines. Results shown represent average relative tumor weights for two to three experiments. When control and drug-treated growth curves were plotted using the log of relative weight versus time, the relationships were nearly linear. Growth of melanoma xenograft 242 was markedly suppressed by BCNU, whereas melphalan and mitomycin were less effective. Melphalan, mitomycin, and BCNU also markedly suppressed growth of melanoma 521. Cisplatin and bleomycin also showed some activity (fig 1).

The response of the ovarian tumors also varied with the drugs tested and tumor lines (fig 2). Tumor T17 was resistant to most drugs. Growth of tumor T17 in mice receiving doxorubicin paralleled that of the control for the first 3 weeks of drug treatment, but by the 4th week (1 week after the cessation of treatment), there was a marked reduction in tumor weight. Ovarian carcinoma 513 was the most drug-responsive tumor studied. Nineteen of 20 (95%) animals treated with melphalan and seven of 19 animals treated with mitomycin showed complete regressions (relative tumor weight < 1.0) and were free of palpable tumor after 5 weeks. In one experiment, animals treated with melphalan were observed for an additional 16 weeks and, in seven of ten (70%), the tumor did not regrow. Cisplatin was also an effective

[5] Abramson IA. A recursive regression algorithm for growth curves, repeated measures and high dimensional models. Manuscript in preparation, 1986.

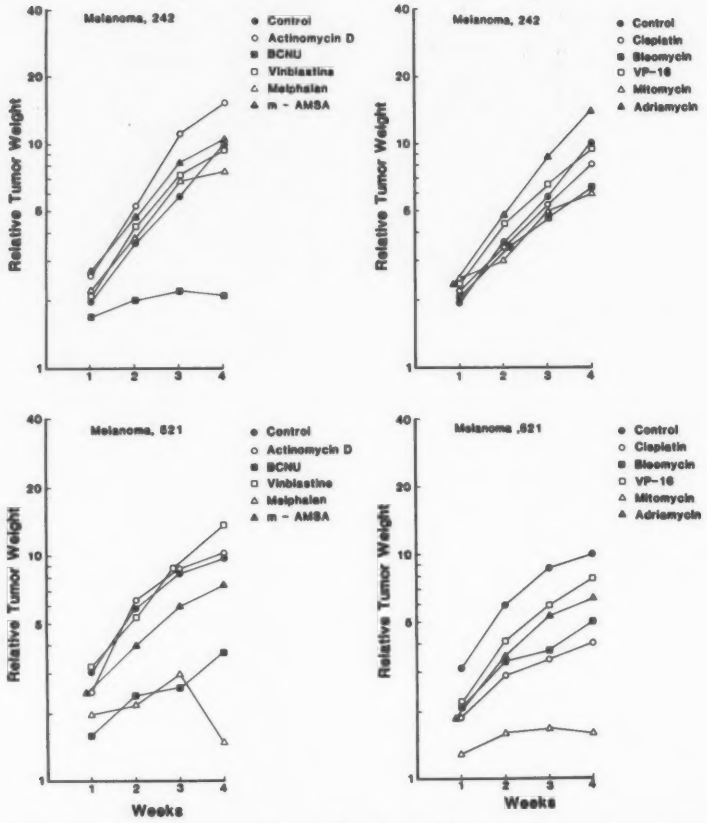


FIGURE 1.—Response of 2 human melanoma xenografts to 10 established chemotherapeutic agents. Groups of 10 animals were treated ip weekly × 4 with 10% less than the LD10 dose. Results represent the means of 2-3 experiments.

inhibitor of 513 growth, and two of 19 (11%) treated mice showed complete tumor regressions.

For ten xenograft tumors tested against ten drugs, table 1 shows percent growth inhibition noted 4 weeks after initiating drug treatment. Results represent the means of two to three experiments using seven to ten animals in each treatment group. Replicate experiments were reproducible. The average variation between experiments as percent growth inhibition was 24% ± 21% ($\bar{x}$ ± SD). When tested against the melanoma xenografts, alkylating agents (BCNU, melphalan, and mitomycin) were the most active drugs. Melphalan and mitomycin were the most active drugs against the three ovarian xenografts, although several other drugs showed some activity. Similar to results reported by others (1,3,11-13), there was variation in response to the same drug among tumors of similar histologic type.

Data Analysis by GDI

Although comparisons of tumor weight on the final day of the experiment provided some measure of in vivo activity, a method for quantitating drug effects over the entire experiment was considered preferable. Growth curves were fitted to regression lines as described above. Correlation coefficients for regression lines ranged from +0.69 to +0.89. For each drug-tumor pair, regression lines were used to derive a GDI (*see* Materials and Methods section). Indices reflecting in vivo drug responses by the seven melanomas and three ovarian xenografts are summarized in table 2. Growth delays are indicated by negative values, while positive numbers indicate treated tumors grew faster than controls. When drugs were ranked according to efficacy against the melanomas, three of the four most active drugs were again alkylating agents (melphalan, mitomycin, and BCNU) and bleomycin was the fourth most active. Thus, had these drugs been studied as unknown compounds, in vivo screening using primary melanoma xenografts would have identified alkylating agents as active.

Only a limited number of ovarian carcinoma xenografts were studied (tables 1 and 2). Tumors T17 and T428 were relatively resistant to most drugs tested.

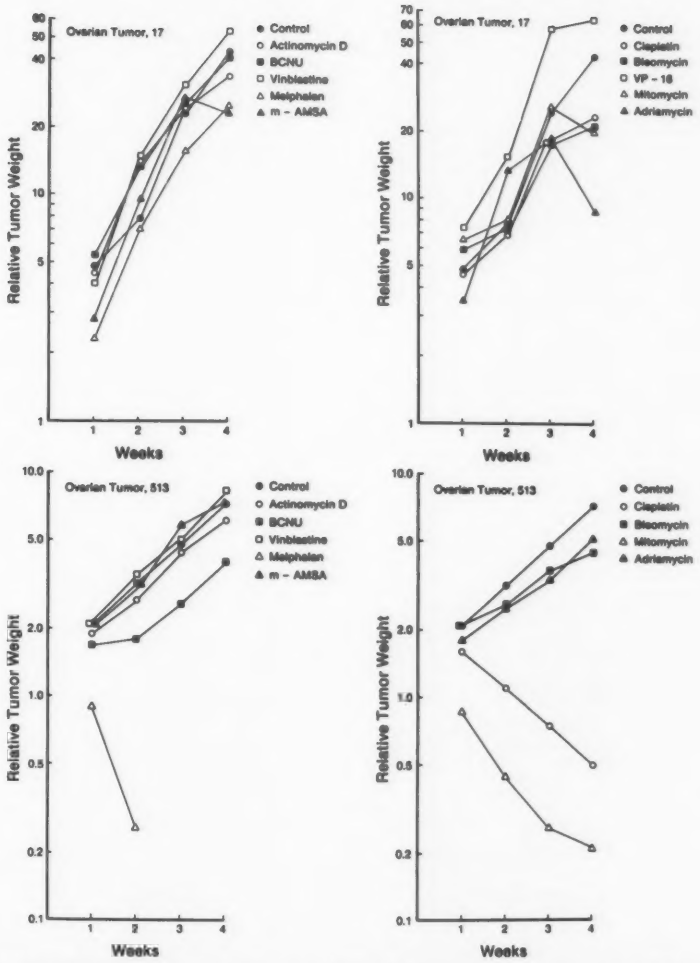


FIGURE 2.—Response of 2 ovarian carcinoma xenografts to 10 established chemotherapeutic agents. Tumor 17 was resistant to most drugs, but tumor 513 showed frank tumor regressions (relative tumor weights < 1.0) with several agents.

However, the most active drug against T17 was doxorubicin (GDI = −4.3, table 2). As noted above, GDIs for tumor T513 reflected dramatic regressions with the alkylating agents melphalan and mitomycin. Overall, doxorubicin, mitomycin, melphalan, and cisplatin appeared to have some activity against the ovarian tumors, but the extent of activity against individual ovarian xenograft tumors varied widely. Thus, while results with melanoma xenografts did not reflect the clinical activity of alkylating agents, three of the drugs most active against ovarian carcinoma xenografts (melphalan, doxorubicin, and cisplatin) have substantial clinical activity in that disease (14).

Correlation Analyses

There were too few studies on any individual tumor to examine effects of tumor passage number on in vivo sensitivities. Overall, for all tumors, there was no apparent relationship between tumor passage number and drug-induced tumor regression. When correlations between tumor growth rate and percent tumor regression were examined (table 3), significant relationships were noted for the aggregate of all drug-tumor pairs and for some drugs alone. With the exception of etoposide, the drugs for which positive relationships were noted (BCNU, melphalan, and mitomycin) were those showing

TABLE 1.—% of control tumor growth 4 wks after start of treatment

Drug	Dose (mg/kg ip weekly × 4)	Tumor: Melanoma 242	354	355	508	521	539	289	Mean*	Ovarian carcinoma 17	428	513	Mean†
Dactinomycin	0.072	150	76	175	94	103	123	97	117	77	145	85	102
BCNU	26.6	21	42	71	58	37	30	62	46	94	101	56	84
Vinblastine	0.76	96	58	109	87	115	89	97	93	123	128	115	122
Melphalan	9.5	75	25	60	39	15	33	48	42	57	51	5	36
m-AMSA	2.5	106	85	129	127	76	116	105	106	53	184	100	112
Cisplatin	3.2	81	63	114	75	40	44	70	70	54	93	7	51
Bleomycin	23.2	64	29	58	84	51	56	80	60	49	83	63	65
Mitomycin	2.4	61	31	17	92	16	54	41	45	49	40	3	31
Doxorubicin	2.6	142	78	73	94	65	101	79	90	20	92	72	61
Etoposide	3.9	96	101	—	75	79	—	—	88	150	—	—	—

*Mean regression for 4-7 melanoma xenografts.
†Mean regression for 3 ovarian carcinoma xenografts.

the greatest activity. This suggests that resistance to drugs such as doxorubicin might have obscured relationships between tumor growth rate and response. Thus, for these xenograft tumors, tumor doubling time was a determinant of in vivo response to some drugs but not others.

DISCUSSION

Nude mouse xenografts have been used to study growth and chemotherapeutic drug sensitivity of human cancers (15) and formed part of the National Cancer Institute drug screening panel from 1975 to the present (6,16). Although their ability to predict new drug efficacy is unknown, they provide an alternative to cell lines for serial propagation of malignant human cells (17). No data are available comparing selective effects of in vitro culture and xenograft propagation.

To examine the potential efficacy of xenografts as preclinical drug screens, we tested ten established anticancer drugs against human melanoma and ovarian carcinomas implanted into athymic mice. The general trend of our results is similar to those obtained in other studies (18-20). Each tumor exhibits a unique drug response profile. Variations in response were most likely due to relative differences in tumor cell drug sensitivity. However, tumor-host relationships for each tumor also differ. The various xenograft tumors differ in their growth rates and tendency to promote reactive fibrosis. We have previously described the variable relationships between tumor size, cell viability, and tumor cell content for individual xenografts (21). Thus, variable drug penetration, tumor cell viability, and tumor vascularity may have affected in vivo drug responses.

Decreases in body weight impair growth of some rodent tumors, and similar effects were observed in human xenografts growing in athymic mice (22,23). Therefore, to compare effects of different drugs, it was necessary to use doses of equivalent toxicity. Previously published data provided only rough guidelines for LD10 doses in BALB/c nude mice (24), and routes of drug administration varied between studies. For these rea-

TABLE 2.—Quantitation of in vivo drug effects by GDI*

	Drug: Dactinomycin	Doxorubicin	*m*-AMSA	BCNU	Bleomycin	Cisplatin	Melphalan	Mitomycin	Vinblastine
				Ovarian carcinoma					
T17	+2.3	−4.3	+1.3	+2.4	−1.8	−1.0	+0.8	−1.9	+4.1
T428	+1.3	+0.1	+3.3	+0.2	−1.0	−1.5	−2.4	−3.7	+2.2
T513	−3.1	−2.5	−1.0	−4.3	−1.7	−8.2	−18.2	−14.3	−1.0
				Melanoma					
T242	+2.3	+1.2	+1.5	−3.4	−0.4	+0.4	−0.1	−0.7	+1.5
T289	−0.4	+0.2	−1.1	−2.9	−1.3	−1.7	−4.4	−3.4	+0.3
T354	−1.6	−0.03	+1.6	+0.02	−2.4	−0.1	−2.1	−4.0	+0.3
T355	+2.7	−1.2	+1.5	−0.1	−1.7	+1.2	−0.9	−6.8	+1.5
T508	+1.0	+0.1	−0.3	−0.4	−0.9	−0.2	−3.5	−0.5	−0.7
T521	+0.3	−1.5	−0.5	−3.3	−2.8	−3.0	−7.3	−5.6	−0.04
T539	+1.2	+0.5	+0.9	−3.9	−1.4	−1.0	−2.1	−0.8	−0.13

*Tumor growth curves were fitted to a quadratic regression model and used to calculate a semiquantitative GDI (*see* Materials and Methods section). Negative values represent drug-induced growth delays.

TABLE 3.—Correlations between in vivo tumor growth delay and tumor doubling times

Drug	N	Correlation r^2	Correlation r	P value*
All	96	0.29	+0.54	< 0.0001
Dactinomycin	10	0	0	NS
Doxorubicin	10	0.04	+0.2	NS
m-AMSA	10	0.14	+0.37	NS
BCNU	10	0.51	+0.71	< 0.05
Bleomycin	10	0.02	+0.14	NS
Cisplatin	10	0.44	+0.66	< 0.05
Melphalan	10	0.39	+0.62	< 0.1
Mitomycin	10	0.42	+0.65	< 0.05
Vinblastine	10	0.03	+0.17	NS
Etoposide	6	0.92	+0.96	< 0.001

*NS = not significant.

sons LD10 doses were determined in non-tumor-bearing mice and equivalent doses of 10% less than this amount used for drug studies. Using these doses and an ip weekly × 4 schedule, average weight losses of treated and control mice did not differ significantly. Further, deaths in drug-treated and control groups were also comparable, indicating the absence of excess, nonspecific drug toxicity.

Most previous studies examined drug activity against a limited number of xenograft tumors or using a limited number of drugs. Further, only a minority of studies used techniques analyzing drug effects over the entire course of the study, rather than at single time points (10). In the present studies, a relatively large number of melanomas were tested and this allowed us to examine the potential performance of these xenografts as a new-drug screen. The fact that our xenografts were established from patients who had not received chemotherapy makes the conclusions potentially more compelling.

When ten drugs active against some human cancer were tested using the melanoma xenografts, a clear pattern of drug activity emerged: the most active drugs were alkylating agents. Since clinical trials have shown alkylating agents to have modest or no activity against melanoma (25), rank-order selection of drugs by xenograft testing does not identify tumor type-specific drugs. Therefore, when used as a new-drug screen, melanoma xenografts may identify the same compounds active in murine leukemia tumors (16). However, each melanoma xenograft had a unique drug sensitivity profile, suggesting tumor heterogeneity which may be useful for detecting novel drug structures.

Testing of ovarian tumor xenografts against the same drugs raised different issues regarding identification of "active" compounds. Although tumors 17 and 428 were resistant to most drugs, doxorubicin had some activity against 17, and 513 showed frank regressions (rather than growth delay) with alkylating agents and cisplatin. These drugs are highly active in untreated ovarian cancer (14), suggesting that only xenograft tumor regressions may be associated with significant clinical activity. More extensive testing of a larger number of ovarian xenografts and prospective evaluation of new agents will be required to answer such questions. These results suggest that more stringent criteria for in vivo activity might identify drugs with clinical activity, and that drug screening using xenografts may be effective for some tumors (ovarian carcinomas), but ineffective for others (melanomas).

Preliminary analyses of factors affecting xenograft tumor growth delay identified tumor growth rate as an important determinant of drug response. This relationship may be pervasive, since some drugs showed little antitumor activity, and this may have obscured correlations between response and growth rate. Thus, selection of rapidly growing tumors by xenografting human tissue could bias responses toward some drugs and not others. Using our data base, a more comprehensive, multivariate analysis of factors influencing in vivo xenograft responses is in progress.

In conclusion, human tumor xenografts established from untreated patients gave rise to tumors with varying patterns of in vivo drug sensitivity. Studies by other investigators suggest this heterogeneity accurately reflects clinical drug responses (26,27). However, as a new-drug screening system, it is unclear what parameters of drug activity identify clinically active compounds or against what types of xenografts. Unless stringent exclusion criteria are established, such systems may not identify clinically active drugs, or target specific tumors for clinical trials, any more efficiently than current rodent tumor screens.

REFERENCES

1. BELLET RE, DANA V, MASTRANGELLO MJ, ET AL. Evaluation of "nude" mouse-human tumor panel as a predictive secondary screen for cancer chemotherapeutic agents. JNCI 63:1185-1188, 1979.
2. BRAAKHUIS BJM, SCHOEVERS ECM, SNEEULOPER G, ET AL. Chemotherapy of human head and neck cancer xenografts with three clinically active drugs: *cis*-platinum, bleomycin and methotrexate. Br J Cancer 48:711-716, 1983.
3. FODSTAD O, AASS H, and PIHL A. Response to chemotherapy of human malignant melanoma xenografts in athymic, nude mice. Int J Cancer 25:453-458, 1980.
4. GIOVANELLA BC, and FOGH J. Present and future trends in investigations with the nude mouse as a recipient of human tumor transplants. *In* The Nude Mouse in Experimental and Clinical Research (Fogh J, and Giovanella B, eds). New York, Academic Press, 1978, pp 281-312.
5. HOUCHENS DP, OVEJERA AA, and BATSEN AD. The therapy of human tumors in athymic (nude) mice. *In* Proceedings of a Symposium on the Use of the Athymic (Nude) Mouse in Cancer Research (Houchens DP, and Ovejera AA, eds). New York, G. Fischer, 1978, pp 267-280.
6. VENDITTI J. Preclinical drug development, rationale and methods. Semin Oncol 8:349-361, 1981.
7. TAETLE R, ROSEN F, HONEYSETT JM, ET AL. Anti-cancer drug screening using nude mouse xenografts (XG). Proc ASCO 4:167, 1985.
8. BEATTIE GM, KNOWLES EF, JENSEN FC, ET AL. Induction of sarcomas in athymic mice. Proc Natl Acad Sci USA 79:3033-3036, 1982.
9. GERAN RI, GREENBERG NH, MACDONALD NM, ET AL. Protocols for screening chemical agents and natural products against animal tumors and other biological systems (third edition). Cancer Che-

mother Rep Part 3, vol 3(2):18-55, 1972.
10. TAETLE R, HOWELL SB, GIULIANI FC, ET AL. Comparison of the activity of doxorubicin analogues using colony-forming assays and human xenografts. Cancer 50:1455-1460, 1982.
11. HOUCHENS DP, OVEJERA AA, RIBLETT SM, ET AL. Human brain tumor xenografts in nude mice as a chemotherapy model. Eur J Clin Oncol 19:799-805, 1983.
12. OSIEKA R, HOUCHENS DP, GOLDIN A, ET AL. Chemotherapy of human colon xenografts in athymic (nude) mice. Cancer 40:2640-2650, 1977.
13. NOWAK K, PECKHAM MI, and STEEL GG. Variation in response of xenografts of colo-rectal carcinoma to chemotherapy. Br J Cancer 37:576-584, 1978.
14. OZOLS RF, and YOUNG RC. Chemotherapy of ovarian cancer. Semin Oncol 11:251-263, 1984.
15. HOUCHENS DP, and OVEJERA AA. Model for human tumor therapy in nude mice. *In* Immune-Deficient Animals (Sordat B, ed). Basel, Karger, 1984, pp 364-369.
16. VENDITTI JM. The National Cancer Institute antitumor drug discovery program, current and future perspectives: a commentary. Cancer Treat Rep 67:767-772, 1983.
17. HISSEN E, WEIZ H, NAUNDORF H, ET AL. Investigations on the instability of drug sensitivity independence on culture and transplantation conditions in case of a human lung carcinoma. Arch Geschwulstforsch 54:443-450, 1984.
18. FRIEDLANDER ML, RUSSELL P, TAYLOR IW, ET AL. Ovarian tumor xenografts in the study of the biology of human epithelial ovarian cancer. Br J Cancer 51:319-333, 1985.
19. GIULIANI FC, ZIRVI KA, and KAPLAN NO. Therapeutic response of human tumor xenografts in athymic mice to doxorubicin. Cancer Res 41:325-335, 1981.
20. BOWEN E, VAN DER VIJGH MM, SCHLIIPER HMM, ET AL. Comparative activity and distribution studies of five platinum analogues in nude mice bearing human ovarian carcinoma xenografts. Cancer Res 45:86-90, 1985.
21. TAETLE R, HONEYSETT JM, ROSEN F, ET AL. Use of nude mouse xenografts as pre-clinical drug screens: further studies on the in vitro growth of xenograft tumor colony forming cells. Cancer 58: 1969, 1986.
22. GOLDIN A. Animal tumor model introduction to the rationale of screening tests. *In* The Design of Clinical Trials in Cancer Therapy (Staquet M, ed). New York, Futura, 1973, pp 7-25.
23. GIOVANELLA BC, SHEPHARD RC, STEHLIN JS, ET AL. Effect of caloric restriction on the growth of human xenografts in nude mice. Proc Am Assoc Cancer Res and ASCO 212:267, 1980.
24. HOUCHENS DP, JOHNSON RK, GASTON MR, ET AL. Toxicity of cancer chemotherapeutic agents in athymic (nude) mice. Cancer Treat Rep 61:103-104, 1977.
25. LUCE JK. Chemotherapy of melanoma. Semin Oncol 2:179-185, 1975.
26. FIEBIG HH, SCHUCHHARDT C, HENSS H, ET AL. Comparison of tumor response in nude mice and in the patients. Behring Inst Mitt 74:343-352, 1984.
27. GIOVANELLA BC, STEHLIN JS, SHEPHARD RC, ET AL. Correlation between response to chemotherapy of human tumors in patients and in nude mice. Cancer 52:1146-1152, 1983.

Phase II Trial of Etoposide in APUD Tumors[1]

David Kelsen,* John Flore, Robert Heelan, Edgar Cheng, and Gordon Magill[2,3]

Thirty-three patients with advanced, metastatic APUD tumors (islet cell, carcinoid, or medullary carcinomas of the thyroid) were treated with etoposide as a single agent. Of 29 evaluable patients, four (14%) had partial responses (95% confidence limits, 1%–26%). Toxic effects seen were those previously reported for etoposide as a single agent. Etoposide has activity in APUD tumors; further studies with this agent are indicated. [Cancer Treat Rep 71:305-307, 1987]

APUD tumors are rare neoplasms which arise in the neural crest; they are characterized histologically by the presence of neurosecretory granules. Malignant carcinoid and islet cell tumors and medullary carcinomas of the thyroid (MCT) comprise a sizable percentage of patients with APUD tumors. Although all three are characterized by a prolonged, indolent course, in which expectant observation, pharmacologic control of diarrhea (or other manifestions of hormone production), and judicious surgery are the mainstays of treatments, tumor progression and increasing symptoms eventually raise the consideration of the use of palliative systemic chemotherapy in some patients. Few chemotherapeutic agents have had adequate trials in these tumors, and even fewer have shown activity. The combination of streptozocin and 5-fluorouracil (5-FU) has been recommended as standard therapy, but response rates vary widely, and toxicity (especially nausea and vomiting) has been substantial (1).

Etoposide is a semisynthetic derivative of podophyllotoxin. The drug is highly active in oat cell carcinomas of the lung, a disease with histologic similarities to other APUD tumors (neurosecretory granules); oat cell tumors also produce a variety of polypeptides (2). Because of its activity in oat cell lung cancer, a phase II trial of etoposide was performed in patients with carcinoid, islet cell, and MCT.

MATERIALS AND METHODS

During the period June 1982 until June 1985, 33 patients with advanced APUD tumors were entered into this trial. All patients had histologic confirmation of disease by the Department of Pathology, Memorial Hospital. In the absence of measurable hormone production or documented primary sites, determination that liver metastases were clearly either from islet cell or from carcinoid tumors was not always possible; these patients were classified as having neuroendocrine tumors, primary unknown. All patients had measurable or evaluable tumor masses, which were assessed by physical examination, chest roentgenogram, or computerized tomographic (CT) scan. Elevated hormonal markers were followed but were not accepted as the only measure of disease; decrease in hormone marker levels without objective tumor mass reduction was not accepted as a major response. The criteria of response for measurable tumor masses were those of Miller et al (3). For patients whose measurable disease involved hepatomegaly, a $\geq$ 50% reduction in hepatomegaly was required. Evaluable disease (tumor masses without clearly defined borders) required independent confirmation of response (marked regression) by a reference radiologist (R. Heelan).

Prior to entrance into the trial, a complete history and physical examination were performed in all patients and the following laboratory studies were obtained: a cbc and platelet count, screening profile, chest roentgenogram, and hormonal markers. These included a 24-hour quantitative urine assay for 5-HIAA (carcinoid tumors); a serum calcitonin level and serum CEA (MCT); and insulin, gastrin, glucagon, or vasoactive intestinal polypeptide levels as appropriate (islet cell tumors). Hormone markers and radionuclide or CT were repeated periodically during the treatment plan.

Etoposide was given as a rapid iv infusion in three divided doses during the first week of each cycle (usually Days 1, 3, and 5). Cycles were repeated every 21

[1] Received June 23, 1986; revised Nov 18, 1986; accepted Dec 5, 1986.

[2] The Gastroenterology Section, Solid Tumor Service, Department of Medicine, and the Diagnostic Radiology Department, Memorial Sloan-Kettering Cancer Center and Cornell University Medical College, New York, NY.

[3] We thank Ms. Robbie Lipperman and Mrs. Ann Martin for their technical assistance.

* *Reprint requests to:* David Kelsen, MD, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021.

days. The starting dosage was 150 mg/m^2/day (total dose, 450 mg/m^2) for previously untreated patients with a Karnofsky performance status (KPS) of $\geq$ 70. For previously treated patients or those with a KPS of 50 or 60, the starting dosage was 120 mg/m^2/day (total dose/cycle, 360 mg/m^2). Etoposide dosages were to be escalated or attentuated by 20 mg/m^2/day (60 mg/m^2/cycle) depending on the degree of myelosuppression.

Informed consent was obtained from all patients.

RESULTS

Of the 33 patients entered into the study, four were inevaluable for response: lost to follow-up before response could be evaluated (three); received concurrent irradiation (one). Patient characteristics of the whole population are shown in table 1. Of note, the median KPS was 70, and 64% of patients had not received prior chemotherapy; 58% had no prior nonsurgical treatment. Sites of measurable or evaluable tumor masses are also shown in table 1. Not surprisingly, the liver was the major site of metastatic disease, although nodal, lung, or subcutaneous lesions were not uncommon.

Hormone production was documented in 12 patients: all three with MCT had elevated calcitonin and CEA values; seven with carcinoid tumors had elevated 5-HIAA values (three also had elevated CEA concentrations); and two patients with islet cell tumors had increased hormone levels (gastrin and glucagon).

Of the 29 evaluable patients, four (14%) had major objective responses (partial remissions of measurable disease or marked regression of evaluable tumor masses): one with MCT, two with bronchial carcinoid, and one with neuorendocrine tumor, primary unknown. One additional patient had a decrease in her 5-HIAA from 246 mg to 75 mg with symptomatic improvement in bone pain; however, her measurable liver metastases decreased by only 10%-20%, so that she was not, by the criteria of this study, considered to be a major responder. Response durations were 170, 288, 343, and 378 days. Two responding patients had received prior chemotherapy.

TABLE 1.—Patient characteristics

Characteristics	No. of patients
Entered	33
Evaluable	29
Median age in yrs (range): 58 (22-76)	
Median performance status (range): 70 (50-90)	
Sex (M:F)	18:15
Prior therapy	
None	19
Chemotherapy	9
Radiation therapy	2
Chemotherapy and radiation therapy	3
Histologic diagnosis	
Carcinoid	17
Islet cell	6
Medullary carcinoma of thyroid	3
Neuroendocrine tumor, primary unknown	7
Sites of metastatic disease	
Liver	22
Lymph nodes	8
Lung	6
Subcutaneous	4
Measurable disease*	19
Evaluable disease*	10

*No. of patients who were evaluable for response.

Toxicity was that usually seen with etoposide. Alopecia was seen in essentially all patients. Nausea and vomiting were mild. Myelosuppression was the major toxicity. The median wbc count nadir was 2800/mm^3 (range, 1000-11,000); the median platelet count nadir was 158,000/mm^3 (range, 80,000-553,000). There were no treatment-related deaths.

DISCUSSION

Many patients with islet cell, carcinoid, or MCT tumors will eventually develop advanced, symptomatic disease. Palliation can be achieved for varying periods using, for example, surgery, embolization, or antidiarrheals, but eventually antineoplastic therapy is frequently considered (4). There are relatively few studies evaluating the use of systemic chemotherapy in neuroendocrine tumors. Single-agent activity (major responses seen in $>$ 15% of patients) has been reported for doxorubicin, streptozocin, 5-FU, and cyclophosphamide (5-7). In a small group of nine patients with carcinoid tumors, interferon caused a marked drop in 5-HIAA levels, but no objective tumor regressions were noted. Several randomized trials have compared single-agent with combination chemotherapy. In an Eastern Cooperative Oncology Group (ECOG) study of patients with carcinoid tumors, 5-FU plus streptozocin was compared to doxorubicin alone (9). There were 86 patients in each arm of the trial. There were no differences in objective response rates (21% versus 22%), in median durations of response (31 weeks versus 26 weeks), or in survival (64 weeks versus 48 weeks). On the other hand, in islet cell tumors, a similar ECOG trial compared streptozocin alone to a combination of streptozocin plus 5-FU in a total of 82 evaluable patients (10). Responses were seen in 15 of 42 patients (36%) receiving streptozocin alone, and in 25 of 40 (63%) receiving the two-drug combination; the difference was statistically significant. The median durations of response were 17 and 24 months, respectively. A third randomized ECOG study compared streptozocin plus 5-FU with streptozocin plus cyclophosphamide in patients with carcinoid tumors (11). A total of 89 evaluable patients were studied (an additional 28 patients were entered into the trial but were inevaluable for response for a variety of reasons). There were no differences in response rates (33% versus 26%) or in durations of response (7 months versus 6 months) or survival (11.2 versus 12.5 months). A number of smaller, single-arm

trials of combination chemotherapy have been reported for either carcinoid or islet cell tumors; there is little evidence that one of these regimens is superior to another, or to the results discussed above. The major toxic effects of all the streptozocin trials are nausea and vomiting (which can be severe), and renal damage. Drug-related deaths were seen in 2%–4% of patients in each of the ECOG studies, usually due to renal toxicity, or to sepsis with myelosuppression. The need to develop newer, less toxic regimens which are hopefully more effective is clear. In this study, etoposide induced major tumor regression in 14% of patients. The toxic effects seen using etoposide in this study were no different than those reported in trials with this agent in other tumors. We conclude that etoposide has activity in APUD tumors, with acceptable toxicity. Further studies of this agent in combination with other drugs are indicated.

REFERENCES

1. BRENNAN M. Cancer of the endocrine system. *In* Cancer: Principles and Practice of Oncology, 2nd ed (DeVita V, Hellman S, and Rosenberg S, eds). Philadelphia, Lippincott Co, 1985, pp 1179–1228.
2. CAVALLI F, GOLDHIRSCH A, and JOSS R. Single agent activity and European experiences with etoposide in the treatment of small cell lung cancer. *In* Etoposide: Current Status and New Developments (Issell B, Muss F, and Carter S, eds). New York, Academic Press, Inc, 1984, pp 163–165.
3. MILLER AB, HOOGSTRATEN B, STAQUET M, ET AL. Reporting results of cancer treatment. Cancer 47:207–214, 1981.
4. MOERTEL C. Treatment of the carcinoid tumor and the malignant carcinoid syndrome. J Clin Oncol 1:727, 1983.
5. SOLOMON A, SONODA T, and PATTERSON FK. Response of metastatic malignant carcinoid tumor to Adriamycin (NSC-123127). Cancer Treat Rep 60:273–276, 1976.
6. MOERTEL CG, LAVIN PT, and HAHN RG. Phase II trial of doxorubicin therapy for advanced islet cell carcinoma. Cancer Treat Rep 66:1567–1569, 1982.
7. BRODER L, and CARTER S. Pancreatic islet cell carcinoma II. Results of therapy with streptozotocin in 52 patients. Ann Intern Med 79:108, 1973.
8. OBERG K, FUNA K, and ALM G. Effects of leukocyte interferon on clinical symptoms and hormone levels in patients with mid-gut carcinoid tumors and the carcinoid syndrome. N Engl J Med 309:129, 1983.
9. ENGSTROM P, LAVIN P, MOERTEL C, ET AL. Streptozotocin plus fluorouracil versus doxorubricin/therapy for metastatic carcinoid tumor. J Clin Oncol 2:1255, 1984.
10. MOERTEL C, HANKEY J, and JOHNSON L. Streptozotocin alone versus streptozotocin plus fluorouracil in the treatment of advanced islet-cell carcinomas. N Engl J Med 303:1189, 1980.
11. MOERTEL C, and HANKEY J. Combination chemotherapy trials in metastatic carcinoid tumors and the malignant carcinoid syndrome cancer chemotherapy trials. Am J Clin Oncol 2:327, 1979.

BRIEF REPORTS

Continuous-Infusion Diaziquone in Acute Myeloid Leukemia: a Southeastern Cancer Study Group Trial[1,2,3]

Gary H. Lyman,[4,*] W. Ralph Vogler,[5] and Marilyn Raney[6]

Diaziquone (AZQ) is a synthetic quinone with considerable activity against L1210 leukemia and potent myelosuppressive activity in man. To test the efficacy and toxicity of AZQ administered by continuous infusion, a phase II multi-institutional trial was undertaken by the Southeastern Cancer Study Group. Eligible adults with acute myeloid leukemia (AML) received AZQ at a dose of 28 mg/m^2 daily by continuous infusion for 5 days. Patients failing to achieve complete remission received a second course utilizing the same dose and schedule. Of 25 evaluable patients with relapsed or refractory AML, three achieved complete response (12%) and two achieved partial response (8%). All patients experienced marked myelosuppression. Severe or life-threatening infection was observed in 15 (56%) patients. Clinical or postmortem evidence of central nervous sytem hemorrhage was encountered in three (12%) patients with severe refractory thrombocytopenia. Minimal nonhematologic toxicity was observed, suggesting that further studies of this agent in combination regimens and possibly for marrow transplantation preparation in patients with acute leukemia are warranted. [Cancer Treat Rep 71:309-310, 1987]

Diaziquone (AZQ) represents a superior analogue of a class of quinones with substantial activity against intraperitoneal L1210 leukemia. Pharmacologic studies in man have demonstrated that AZQ administration is associated with a dose-independent plasma half-life of approximately 30 minutes (1). In early clinical trials utilizing intermittent schedules of administration, AZQ demonstrated potent myelosuppressive activity in man, with minimal nonhematologic toxicity (2-4). Phase II studies utilizing intermittent schedules of AZQ administration have generally failed to demonstrate activity against human acute luekemia, however (5-7). In vitro studies have suggested that the antitumor effect of AZQ may correlate with the duration of exposure (8). A recent clinical trial has demonstrated activity for AZQ administered by continuous infusion in adults with relapsed acute myeloid leukemia (AML) (9). The phase II trial reported here was undertaken to further assess the schedule dependency of AZQ on remission induction and toxicity in AML.

METHODS

Adults with previously treated AML, including acute myeloblastic, acute monocytic, acute myelomonocytic, acute promyelocytic, and acute erythroleukemia, were eligible for study. Patients with at most one induction effort following relapse or a maximum of two induction efforts were eligible regardless of performance status. Patients with more than two induction efforts following relapse were required to have a Karnofsky performance status of at least 50% and an anticipated survival of ≥ 1 month. Eligible patients were required to have recovered from the hematologic effects of prior therapy. Complete response was defined as a normocellular bone marrow with $< 5\%$ blasts and normal hematopoiesis. A partial response was defined as a normocellular marrow with $< 5\%$ blasts and $< 30\%$ blasts and promyelocytes. Following informed consent, eligible patients were started on an induction regimen consisting of AZQ administered at a dose of 28 mg/m^2 daily by continuous

[1] Received July 16, 1986; revised Sept 23, 1986; accepted Oct 8, 1986.
[2] Supported by Public Health Service grants CA-19657, CA-24456, CA-03013, CA-03177, CA-05634, CA-03227, CA-03376, CA-12223, CA-13237, CA-15584, CA-28169, CA-23909, CA-28171, and CA-37577 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.
[3] Presented in part at the annual meeting of the American Society of Hematology, New Orleans, LA, Dec 1985.

[4] H. Lee Moffitt Cancer Center and Research Institute at the University of South Florida, Tampa.
[5] Leukemia Research Laboratory, Emory University, Atlanta, GA.
[6] Statistical Center, Southeastern Cancer Study Group, Birmingham, AL.
* *Reprint requests to:* Gary H. Lyman, MD, MPH, Division of Medical Oncology, Department of Internal Medicine, University of South Florida, College of Medicine, 12901 N 30th St, Box 19, Tampa, FL 33612.

infusion for 5 days. Patients failing to achieve complete remission received a second 5-day course of AZQ using the same dose and schedule. Patients achieving complete remission were scheduled to receive two consolidation courses consisting of AZQ at a dose of 18 mg/m^2/day administered by 2-hour infusion for 5 days.

RESULTS

Twenty-eight patients with AML were registered on this study with the patient characteristics shown in table 1. The mean age of patients entered on study was 45 ± 3.0 (SEM) years. Patients had received a median of three previous remission induction efforts. Of the 20 patients who had previously achieved complete remission, 11 had failed subsequent induction efforts prior to entry on this study.

Twenty-five patients were evaluable for response. Inevaluable patients included two with insufficient data and one who died within 2 days of treatment initiation. Complete hematologic remission was observed in three patients (12%; 95% confidence limits [0%, 25%]). Five patients (20%) had either partial or complete response (95% confidence limits [8%, 42%]). One of seven evaluable patients (14%) who had failed all previous induction efforts achieved complete remission. Of the 18 evaluable patients who had previously achieved complete remission, two (11%) achieved complete remission with AZQ, while two (11%) had partial response. Two patients achieving complete remission received consolidation therapy. Complete remissions lasted 6, 12, and 12+ weeks, while partial remissions lasted 4 and 6 weeks.

TABLE 1.—Patient characteristics

Characteristics	No. of patients (%)
Sex	
Male	15 (54)
Female	13 (46)
Race	
White	24 (86)
Black	4 (14)
Age	
< 40	7 (25)
40-60	14 (50)
> 60	7 (25)
Prior remissions	
0	8 (29)
1	11 (39)
2	6 (21)
3	3 (11)
Evaluable for response	
No	3 (11)
Yes	25 (89)
Treatment response	
CR	3 (12)
PR	2 (8)
None	20 (80)
Severe toxic effects	
Marrow	28 (100)
Hemorrhage	4 (14)
Infection	14 (50)
Hepatic	3 (9)
Gastrointestinal	1 (3)
Neurologic	3 (9)

Of the 20 patients failing to achieve remission, all attained marrow hypocellularity. Seven patients experienced regrowth of leukemia within 4 weeks, while 13 demonstrated persistent hypocellularity beyond 4 weeks. Eight patients died with persistent hypoplasia ranging in duration from 4 to 9 weeks; all eight had achieved complete remission with previous regimens. Patients dying on study had a median duration of aplasia of 4 weeks.

Toxicity was predominately hematologic, with all patients exhibiting marked marrow aplasia. Clinical or postmortem evidence of central nervous system hemorrhage was encountered in three patients with refractory severe thrombocytopenia. Nonhematologic toxic effects were generally mild and not life-threatening.

DISCUSSION

AZQ administered by continuous infusion has demonstrable activity in patients with refractory and relapsed AML, suggesting a schedule dependency for this agent. Marrow aplasia was observed in all patients, with minimal nonhematologic toxicity. While the cases of central nervous system hemorrhage are of concern in light of the pharmacology of AZQ, each patient was at extraordinary risk by virtue of refractory thrombocytopenia. The potential value of this agent in less heavily treated patients with AML and in combination with other active agents appears sufficient to warrant further clinical investigation. Studies to assess the value of AZQ as preparation for marrow transplantation in patients with acute leukemia may also be warranted.

REFERENCES

1. BACHUR NR, COLLINS JM, KELLY JA, ET AL. Diaziquone in plasma and cerebrospinal fluid. Clin Pharmacol Ther 31:650-655, 1982.
2. BEDIKIAN AY, BODEY GP, BURGESS MA, ET AL. Phase I study of aziridinylbenzoquinone. Cancer Clin Trials 4:459-463, 1981.
3. GRIFFIN JP, NEWMAN RA, MCCORMACK JJ, ET AL. Clinical and clinical pharmacologic studies of aziridinylbenzoquinone. Cancer Treat Rep 66:1321-1325, 1982.
4. CAREY RW, COMIS RL, ANBAR D, ET AL. Cancer and Leukemia Group B phase II non-small cell lung carcinoma trial: aziridinylbenzoquinone (AZQ). Cancer Treat Rep 67:95-96, 1983.
5. VAN ECHO DA, SCHULMAN P, BUDMAN DR, ET AL. A phase I trial of aziridinylbenzoquinone (NSC-182986) in patients with previously treated acute leukemia. Am J Clin Oncol 5:405-415, 1982.
6. ETTINGER LJ, SIEGEL SE, and BELASCO JD. Phase I study of aziridinylbenzoquinone (AZQ) in children with leukemia. Proc Am Soc Hematol 60:155a, 1982.
7. WALTERS RS, KEATING MJ, and BODEY GP. Phase I-II study of aziridinylbenzoquinone in acute leukemia. Proc ASCO 1:134, 1982.
8. EGORIN MJ, FOX BM, SPIEGEL JF, ET AL. The cellular pharmacology of aziridinylbenzoquinone (AZQ, NSC-182986). Proc Am Assoc Cancer Res 23:696, 1982.
9. LEE EJ, VAN ECHO DA, EGORIN MJ, ET AL. Diaziquone given as a continuous infusion is an active agent for relapsed adult acute nonlymphocytic leukemia. Blood 1:182-187, 1976.

BRIEF REPORTS

Phase II Study of Carboplatin in Previously Untreated Patients With Metastatic Small Cell Lung Carcinoma[1,2]

Renee H. Jacobs, Jacob D. Bitran,* Margaret Deutsch, Philip C. Hoffman, Joseph Sinkule, Sandra Purl, and Harvey M. Golomb[3]

Fourteen previously untreated patients with metastatic small cell lung carcinoma (SCLC) were treated with carboplatin, 400 mg/m^2, given as a 1-hour infusion every 28 days. Eleven patients had a partial response to therapy [response rate, 79% (95% confidence limits, 57%-100%)]. The median duration of response was 8 weeks (range, 4-41) and the median survival was 29 weeks (range, 11-68+). Hematologic toxicity was limited to one patient who developed World Health Organization (WHO) grade IV thrombocytopenia; no patient developed infection due to granulocytopenia. Nonhematologic toxicity was limited to WHO grade I and II nausea and vomiting. This study demonstrates that carboplatin is a highly active drug in SCLC with minimal toxicity. Future studies should evaluate its use in combination with other chemotherapeutic drugs in SCLC. [Cancer Treat Rep 71:311-312, 1987]

Systemic chemotherapy is the treatment of choice for patients with metastatic small cell lung carcinoma (SCLC). Many chemotherapeutic drugs are active in SCLC, giving response rates up to 80% when used in combination (1,2). In spite of the high response rate, only 5%-15% of patients with limited SCLC survive beyond 5 years, and patients with extensive disease are very rarely cured (3-5).

Carboplatin (CBDCA) is a second-generation platinum analog which is of clinical interest because of the lack of nephrotoxicity (6-8). Smith et al (9) have reported a 41% overall response rate with CBDCA in 56 patients with SCLC. Their response rate in 30 previously untreated patients with stage III SCLC was 60%. The objective of this trial was to evaluate the activity of CBDCA in previously untreated patients with extensive (metastatic) SCLC.

PATIENTS AND METHODS

From August 1984 through December 1985, 14 previously untreated patients with histologically proven extensive SCLC were treated with CBDCA. Patients with limited SCLC with only supraclavicular nodal involvement or pleural effusion as the sole site of metastatic disease were ineligible. In addition, patients with an Eastern Cooperative Oncology Group performance status > 3 or a creatinine clearance < 40 ml/min were ineligible.

Prior to entry all patients required a complete history and physical examination, urinalysis, cbcs, chemistry profile (including renal and liver function tests), chest x-ray, ECG, creatinine clearance, bone scan, computerized tomographic (CT) scan of the brain, gallium-67 scan, and CT scan of the chest and upper abdomen to the level of the adrenal glands. Visceral metastases required confirmation by scintographic or radiologic studies.

CBDCA was supplied by the Investigational Drug Branch at the National Cancer Institute in 20-ml amber vials with 150 mg of CBDCA prepared as a white lyophilized powder with 150 mg of mannitol. The drug was reconstituted in sterile water. The dose of CBDCA was 400 mg/m^2 given iv as a 1-hour infusion and repeated every 28 days. Hydration was not required with administration of the drug.

Patients were evaluated for response following two courses of CBDCA. A complete response (CR) was defined as the disappearance of all measurable and evaluable lesions; a partial response (PR) was a $> 50\%$ reduction in

[1] Received Aug 6, 1986; revised Nov 7, 1986; accepted Nov 17, 1986.
[2] Supported by Goldfine-Smilgoff Cancer Research Organization and the Gatzert Fund.
[3] Department of Medicine, University of Chicago Medical Center and Michael Reese Medical Center, IL.
* *Reprint requests to:* Jacob D. Bitran, MD, Michael Reese Medical Center, Lake Shore Drive at 31st St, Chicago, IL 60616.

TABLE 1.—Patient characteristics and results

Total No. of patients	14
Male	6
Female	8
Median age (yrs)	58
Range	36–78
Median performance status	1
Median courses of treatment	3
Range	2–11
Response (%)	
CR	0
PR	11 (79)
No response	3 (21)
Median duration of response (wks)	9
Range	4–41
Median survival (wks)	29
Range	11–68+

the greatest tumor diameter and its perpendicular for all measurable lesions.

Patients were removed from study if they received two courses of CBDCA and had no response to therapy (mixed response, stable disease, or progressive disease were scored as no response). Patients with a CR or PR were continued on study until disease progression occurred. Patients developing a creatinine clearance < 40 ml/min or ototoxicity were taken off study. Patients taken off study were treated with either radiotherapy alone or combination chemotherapy with methotrexate, cyclophosphamide, doxorubicin, and vincristine (MOCA) as secondary therapies.

RESULTS

Response to Therapy and Toxicity

Patient characteristics are summarized in table 1. All 14 patients were evaluable for response. A PR was seen in 11 of 14, or 79% of patients (95% confidence limits of 57%–100%); one patient had a mixed response and two patients had progressive disease. There was no association demonstrated between the site of metastatic disease and the response to therapy. The median duration of response was 8 weeks (range, 4–41). Two of 14 patients are still undergoing therapy with CBDCA. Twelve patients developed progressive disease on CBDCA and were treated with radiotherapy alone (six) or MOCA (six). Of the six patients treated with MOCA, three achieved a PR (50%). The median duration of PR was 15 weeks. The median survival was 29 weeks (range, 11–68+).

The toxic effects of therapy were generally mild. Mild to moderate nausea and vomiting was observed in 12 (86%) of 14 patients. One patient developed a 50% decrease in creatinine clearance (66 ml/min to 32 ml/min) after three courses of CBDCA. The etiology of the decline in renal function is unclear; however, the patient was known to have diabetes mellitus and atherosclerotic cardiovascular disease. Alopecia occurred in 29% of patients. Other nonhematologic toxic effects such as ototoxicity, neurotoxicity, and mucositis were not observed. Hematologic toxicity was acceptable; the median nadir wbc count was 4800/μl (range, 1500–7200) and the median nadir platelet count was 164,000/μl (range, 19,000–394,000). Three patients had absolute granulocyte counts $< 1000/\mu$l which were not complicated by fever or infection. Only one patient had a platelet count $< 20,000/\mu$l without clinical evidence of bleeding.

DISCUSSION

This study demonstrates that CBDCA is an active drug in SCLC, and supports the earlier results by Smith et al (9). Although myelosuppression was found to be the dose-limiting toxic effect in phase I studies of previously treated patients, it was not a significant toxic effect encountered in our group of previously untreated patients (7,8). Unlike cisplatin, nonhematopoietic toxic effects such as nausea, vomiting, and nephrotoxicity were reduced. This has been further established in a prospective randomized trial in ovarian carcinoma comparing CBDCA and cisplatin, which demonstrated a statistically significant reduction in nephrotoxicity, gastrointestinal toxicity, subclinical ototoxicity, treatment delay, and discontinuation of therapy (10).

CBDCA is an effective drug in SCLC, free of many of the toxic effects associated with cisplatin therapy, and should be studied in combination with other active drugs in this disease.

REFERENCES

1. WEISS RB. Small cell carcinoma of the lung: therapeutic management. Ann Intern Med 88:522–531, 1978.
2. AISNER J, ALBERTO P, BITRAN J, ET AL. Role of chemotherapy in small cell lung cancer: a consensus report of the International Association for the Study of Lung Cancer workshop. Cancer Treat Rep 67:37–43, 1983.
3. LIVINGSTON RB, STEPHENS RL, BONNET JD, ET AL. Long-term survival and toxicity in small cell lung cancer. Am J Med 77:415–417, 1984.
4. JOHNSON BE, IHDE DC, BUNN PA, ET AL. Patients with small-cell lung cancer treated with combination chemotherapy with or without irradiation. Ann Intern Med 103:430–438, 1985.
5. JACOBS RH, GREENBURG A, BITRAN JD, ET AL. A ten year experience with combined modality therapy for Stage III small cell lung carcinoma. Cancer. In press.
6. KOELLER JM, EARHART RH, DAVIS TE, ET AL. Phase I trial of carboplatin (NSC 241240) by bolus injection. Proc Am Assoc Cancer Res 24:162, 1983.
7. LASSUS M, OHNUMA T, LEYVRAZ S, ET AL. A phase I study of CBDCA (carboplatin). Proc ASCO 2:37, 1983.
8. VAN ECHO DA, EGORIN MJ, WHITACRE MY, ET AL. Phase I clinical and pharmacologic trial of carboplatin daily for 5 days. Cancer Treat Rep 68:1103–1114, 1984.
9. SMITH IE, HARLAND SJ, ROBINSON BA, ET AL. Carboplatin: a very active new cisplatin analog in the treatment of small cell lung cancer. Cancer Treat Rep 69:43–46, 1985.
10. CANETTA R, ROZENCWEIG M, and CARTER SK. Carboplatin: the clinical spectrum to date. Cancer Treat Rev 12(suppl A):125–136, 1985.

BRIEF REPORTS

Percutaneous Hepatic Arterial Infusion of Cisplatin for Metastatic Breast Cancer[1]

Giuseppe Fraschini,[2,*] Gary Fleishman,[3] Hwee-Yong Yap,[4] C. Humberto Carrasco,[2] Chusilp Charnsangavej,[2] Yehuda Z. Patt,[2] and Gabriel N. Hortobagyi[2,5]

We treated 31 patients with breast cancer metastatic to the liver and refractory to prior chemotherapy with percutaneous hepatic arterial infusion of cisplatin (DDP) (120 mg/m^2) at 4-week intervals. Partial responses occurred in five of 26 evaluable patients, with a median time to disease progression of 15+ weeks (range, 8+ to 55) and a median duration of survival of 11 months (range, 8–22). Toxic effects were acceptable and, except for catheter-related complications, were essentially similar to those encountered when DDP was given iv. Percutaneous hepatic arterial infusion of DDP has modest activity in the treatment of breast cancer metastatic to the liver. [Cancer Treat Rep 71:313-315, 1987]

Iv administered cisplatin (DDP) has shown modest activity against breast cancer. Responses were seen only in soft tissue or lung metastases, with considerable gastrointestinal, renal, or neurological side effects (1-3).

The therapeutic efficacy of DDP in regionally confined malignancies may improve with local delivery (4,5). In clinical pharmacology studies, the intra-arterial administration of DDP resulted in an increased exposure of the tumor to the drug within the target organ (6,7).

The favorable initial clinical and pharmacologic reports prompted us to explore the therapeutic efficacy of hepatic arterial infusion with DDP in patients with metastatic breast cancer who had failed systemic therapy and in whom the bulk of the disease was confined to the liver.

PATIENTS AND METHODS

Between February 1978 and December 1983, we treated 31 breast cancer patients with hepatic arterial infusion of DDP. The patients were registered on protocol with the institutional central Data Management Office.

Patients with only or predominantly liver metastases refractory to prior systemic chemotherapy were eligible. Other eligibility criteria included normal renal function, Zubrod performance status of 3 or better, and written informed consent.

All patients were admitted to the hospital for the treatment procedure. Percutaneous catheters were newly introduced with each treatment by the Seldinger technique (8), either into the femoral, axillary, or high brachial arteries. The catheter was selectively positioned into the proper or common hepatic artery.

DDP was infused at a starting dose of 120 mg/m^2 diluted in 500–1000 ml of normal saline and mixed with 3000 units of sodium heparin. The patients also received the usual iv hydration. The DDP doses were increased or decreased by 20% in subsequent courses depending on the status of the renal function and the degree of myelosuppression. Courses of therapy were repeated on an average of 4-week intervals.

Response to therapy was assessed in accordance with World Health Organization criteria (9) and based on the consensus of a panel of at least three experienced radiologists and oncologists.

RESULTS

Five of 31 patients entered in our study were not evaluable. Two patients were lost to follow-up after the

[1] Received Aug 8, 1986; revised Nov 3, 1986; accepted Nov 20, 1986.

[2] Departments of Medical Oncology (*G. Fraschini* and *G. N. Hortobagyi*), Radiology (*C. H. Carrasco* and *C. Charnsangavej*), and Clinical Immunology (*Y. Z. Patt*), The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

[3] Hematology-Oncology Associates of Houston, TX.

[4] Cancer Screening and Treatment Centre, Singapore, Republic of Singapore.

[5] We thank Theresa Criswell for her assistance in preparing the manuscript.

* *Reprint requests to:* Giuseppe Fraschini, MD, Department of Medical Oncology, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, Houston, TX 77030.

TABLE 1.—Pretreatment characteristics of 26 evaluable patients

Characteristic	
Median age in yrs (range)	47 (28–66)
Median disease-free interval in mos (range)	19 (0–84)
Median Zubrod performance status (range)	1 (0–3)
Median duration of prior therapy in mos (range)	23 (3–49)
Median No. of prior regimens (range)	2 (1–7)
No. of patients	
Premenopausal	17
Postmenopausal	9
No. of metastatic sites	
Liver only	13
Liver and others	13

TABLE 2.—Toxicity in 29 patients receiving intrahepatic DDP

Type	% of patients
Catheter-related	
Mild bleeding at insertion site	14
Hematoma at insertion site	10
Hepatic artery intimal tear	14
Hepatic arterial occlusion	10
Chemotherapy-related	
Nausea and emesis	97
Fatigue, malaise	28
Neutropenic fever	21
Decrease of creatinine clearance to 35–50 ml/min	10
Mild ototoxicity	7
Gastroduodenitis	3
Myelosuppression	
Median lowest granulocyte count, cells/mm^3 (range): 1700 (200–4300)	
Median lowest platelet count, $\times 10^3$ cells/mm^3 (range): 105 (14–212)	

first course and were not evaluable for toxicity or response. One patient refused further therapy following the first course, which had caused no significant toxicity. The fourth patient concomitantly received other systemic chemotherapy. The last patient died of an unrelated cerebral vascular accident after the first course.

The pretreatment characteristics of the 26 evaluable patients are shown in table 1. All patients but three had previously received doxorubicin-containing regimens. All extrahepatic sites of disease were of small tumor burden and quite stable. The evaluable patients received a median of two courses (range, one to six).

Five patients (19%; 95% confidence interval, 7%–38%) with well-defined nodular lesions, clearly measurable by angiography and computed tomography, achieved partial remission of their disease.

The median time to disease progression was 15+ weeks (range, 8+ to 55), and the median survival was 11 months (range, 8–22).

The five responding patients had pretreatment performance scores of either 0 or 1, and all but one had received only one chemotherapy regimen. Additionally, none had measurable extrahepatic disease. Two patients who did not respond in the liver also developed new metastatic sites in the brain and pleura, respectively.

The toxic effects that occurred are shown in table 2. Nausea and emesis, often severe, fatigue, and myelosuppression with fever, which required hospitalization for parenteral antibiotic coverage, decreased the treatment tolerance. However, most patients could tolerate the protocol dosage. Average initial and final doses were 120 and 115 mg/m^2, respectively.

DISCUSSION

Even though the antitumor effect of DDP in a number of solid tumors is well-recognized, its role in the systemic therapy of metastatic breast cancer appears limited. At doses of 100 mg/m^2 we observed no responses (1) and others reported remissions in 12% of the patients (2). With 120 mg/m^2, 21% of the patients responded in an additional study, but the toxicity was considerable (3). With the exception of one response in the lung, all responses occurred in soft tissue disease.

The rationale for using DDP by hepatic arterial infusion is not conclusively supported by clinical pharmacology studies (6,7) and has recently been criticized (10). Pharmacology data to support the theoretical advantage were not available in our patients. It should be noted, though, that most clinical trials with intra-arterial chemotherapy have reported some advantage for this route of delivery, irrespective of pharmacokinetic considerations (11).

The results obtained in our trial with intra-arterial DDP appear more significant if we consider that refractory liver metastases respond poorly to second-line chemotherapy.

However, a comparison of our results with those obtained with systemic DDP trials is not possible in view of the uneven patient populations. Additionally, we could not document any significant palliation or improvement in quality of life in our patients. The toxicity observed was relevant and the responding patients had good performance and few symptoms at the start.

The myelosuppressive effect was not different from that seen in patients treated with iv DDP. This may be explained by the different patient characteristics and does not necessarily prove that the hepatic extraction of the drug was minimal. In addition, clinical pharmacology has shown that intra-arterial administration of DDP does not significantly decrease systemic drug exposure, although it may increase regional exposure (6,7).

In summary, our experience suggests that intra-arterial DDP has modest activity against hepatic metastases of breast carcinoma.

REFERENCES

1. YAP H-Y, SALEM P, HORTOBAGYI GN, ET AL. Phase II study of *cis*-dichlorodiammineplatinum(II) in advanced breast cancer. Cancer Treat Rep 62:405–408, 1978.

2. OSTROW S, EGORIN M, AISNER J, ET AL. High-dose *cis*-diamminedichloroplatinum therapy in patients with advanced breast cancer: pharmacokinetics, toxicity and therapeutic efficacy. Cancer Clin Trials 3:23–27, 1980.
3. FORASTIERE AA, HAKES TB, WITTES JT, ET AL. Cisplatin in the treatment of metastatic breast carcinoma: a prospective randomized trial of two dosage schedules. Am J Clin Oncol 5:243–247, 1982.
4. CALVO DB, PATT YZ, WALLACE S, ET AL. Phase I–II trial of percutaneous intra-arterial *cis*-diamminedichloroplatinum(II) for regionally confined malignancy. Cancer 45:1278–1283, 1980.
5. CHUANG V, WALLACE S, BENJAMIN R, ET AL. Therapy of osteosarcoma by intra-arterial *cis*-platinum and limb preservation. Cardiovasc Intervent Radiol 4:229–235, 1981.
6. KELSEN DP, HOFFMAN J, ALCOCK N, ET AL. Pharmacokinetics of cisplatin regional hepatic infusions. Am J Clin Oncol 5:173–178, 1982.
7. STEWART DJ, BENJAMIN RS, ZIMMERMAN RM, ET AL. Clinical pharmacology of intraarterial *cis*-diamminedichloroplatinum(II). Cancer Res 43:917–920, 1983.
8. SELDINGER SI. Catheter replacement of the needle in percutaneous arteriography: a new technique. Acta Radiol 39:368–376, 1953.
9. MILLER AB, HOOGSTRATEN B, STAQUET M, ET AL. Reporting results of cancer treatment. Cancer 47:207–214, 1981.
10. CAMPBELL TN, HOWELL SB, PFEIFLE CE, ET AL. Clinical pharmacokinetics of intraarterial cisplatin in humans. J Clin Oncol 1:755–762, 1983.
11. STEPHEN FO. Selective embolization and organ perfusion with cytostatics. Pharmacokinetics of intraarterial chemotherapy. *In* Recent Results in Cancer Research (Schwlemme K, and Aligner V, eds). 1983, vol 86, pp 1–12.

BRIEF REPORTS

Pharmacokinetic Comparison of Intranasal, Oral, and Intramuscular Metoclopramide in Healthy Volunteers[1,2]

Marc L. Citron,[3,4,*] John R. Reynolds,[3,5] JagMohan Kalra,[4,6] Bruce G. Kay,[3,5] Kim A. Nathan,[3] Norman D. Jaffe,[3] and Frederic R. Miller[3]

This was a randomized, crossover study of the bioavailability and pharmacokinetics of metoclopramide given by intranasal (IN), oral (PO), and intramuscular (IM) routes. The formulations tested were 5 and 10 mg of IN gel, 10 mg PO, and 5 mg IM. The findings showed that metoclopramide follows similar absorption and elimination characteristics when given via these three extravascular routes. There were no statistically significant differences in the area under the drug concentration versus time curve (AUC) or peak metoclopramide plasma concentration (C_{max}) data following PO, IM, or 10-mg IN administration. However, the 5-mg IN doses achieved an AUC that was 39.5% the AUC of the 10-mg IN dose. These data show consistent and relatively predictable metoclopramide plasma concentrations following IN administration. Further, 10-mg doses of IN and PO metoclopramide appear to be bioequivalent. [Cancer Treat Rep 71:317-319, 1987]

Previous clinical trials have established that metoclopramide is an effective antiemetic agent, especially for patients with chemotherapy-induced nausea and vomiting (1,2). Administration of this agent is currently limited to oral (PO) and parenteral routes. However, once vomiting has begun, these routes of administration may not be practical in some clinical settings.

Recently, a formulation of metoclopramide has been developed that permits intranasal (IN) administration. Preclinical studies in animals showed that IN metoclopramide resulted in significant systemic drug absorption (A. Hussain and Y. Aramaki, unpublished data). This paper reports a randomized, crossover study of the bioavailability and pharmacokinetics of metoclopramide given via IN, PO, and intramuscular (IM) routes.

METHODS

Subjects.—The subjects were eight healthy males > 18 years of age, who had physical examinations, laboratory parameters, and electrocardiograms within the normal range. Informed consent approved by the Medical Center Human Studies Subcommittee was obtained for all subjects.

Study design.—This was a randomized, four-way crossover study, in which each subject received a single dose of metoclopramide on four occasions, separated by 1-week washout periods. On each occasion, after an overnight fast, one of the following formulations of drug was administered: 10-mg IN gel (10 mg/0.1 ml), 5-mg IN gel (5 mg/0.1 ml), 10-mg tablet (Reglan), or 5-mg IM injection (Reglan).

Metoclopramide plasma concentrations.—Venous blood samples were collected in plain, red-top tubes at baseline and according to the following schedules: 15, 30, 60, 90, 120, 150, 180, 240, 360, and 480 minutes after PO administration; and 5, 10, 20, 30, 45, 60, 120, 180, 240, 360, and 480 minutes after IN or IM administration. Blood samples were centrifuged, and the plasma was frozen at −20°F. Metoclopramide plasma concentrations were determined using a high-performance liquid chromatography technique.

Computations.—The area under the drug concentration versus time curve (AUC) from 0 to 480 minutes was

[1] Received Oct 11, 1985; revised Nov 10, 1986; accepted Nov 24, 1986.
[2] Supported by a grant from Nastech Pharmaceutical Co, Hauppauge, NY.
[3] Division of Hematology/Oncology (*M. L. Citron, K. A. Nathan,* and *N. D. Jaffe*), Clinical Pharmacokinetics (*J. R. Reynolds*), Pharmacy Services (*B. G. Kay*), and Department of Medicine (*F. R. Miller*), Long Island Jewish Medical Center, New Hyde Park, NY.
[4] State University of New York at Stony Brook, NY.
[5] College of Pharmacy, St. John's University, Jamaica, NY.
[6] Queens Hospital Center affiliation of the Long Island Jewish Medical Center, Jamaica, NY.
**Reprint requests to:* Marc L. Citron, MD, Division of Hematology/Oncology, Long Island Jewish Medical Center, New Hyde Park, NY 11042.

TABLE 1.—Bioavailability and pharmacokinetics of metoclopramide*

Parameter	10 mg, PO	5 mg, IM	5 mg, IN	10 mg, IN
AUC (ng/ml · min)	9614 ± 1508	9934 ± 945	4023 ± 1512	10,198 ± 1240
C_{max} (ng/ml)	36.0 ± 7.8	34.0 ± 3.3	15.2 ± 5.4	35.1 ± 7.6
T_{max} (min)	97.5 ± 21.2	91.9 ± 39.6	80.6 ± 36.7	131.3 ± 39.1
Kel ($min^{-1} \times 10^3$)	1.89 ± 1.13	2.67 ± 1.00	2.59 ± 1.41	2.77 ± 1.26
T½ (min)	367 ± 219	260 ± 97	267 ± 145	250 ± 114

*Values = mean ± SD.

calculated using the trapezoidal rule (3). Peak metoclopramide plasma concentrations (C_{max}) and time to peak (T_{max}) were obtained directly from the raw data. The elimination rate constants (Kel) were derived by fitting least-squares lines to the natural log concentration versus time data, starting with the plasma concentration that occurred at least 1 hour after the observed peak. Half-life (T½) was derived by dividing 0.693 by Kel.

Statistical methods.—Analysis of variance (ANOVA) was used to compare the mean values for AUC, C_{max}, T_{max}, and T½. Statistical significance was determined at the 5% level.

RESULTS

The results for AUC, C_{max}, T_{max}, Kel, and T½ are listed in table 1 and are graphically represented in figure 1. No significant differences ($P < 0.05$) in the AUC data were observed following PO, IM, and 10-mg IN doses; the 10-mg IN dose had an AUC 3% greater than that of the PO dose and 6% greater than that of the IM dose. The 5-mg IN dose showed a significantly smaller AUC ($P < 0.05$), approximately 39.5% that of the 10-mg IN formulation. In addition, no statistically significant differences were found in the C_{max} data following PO,

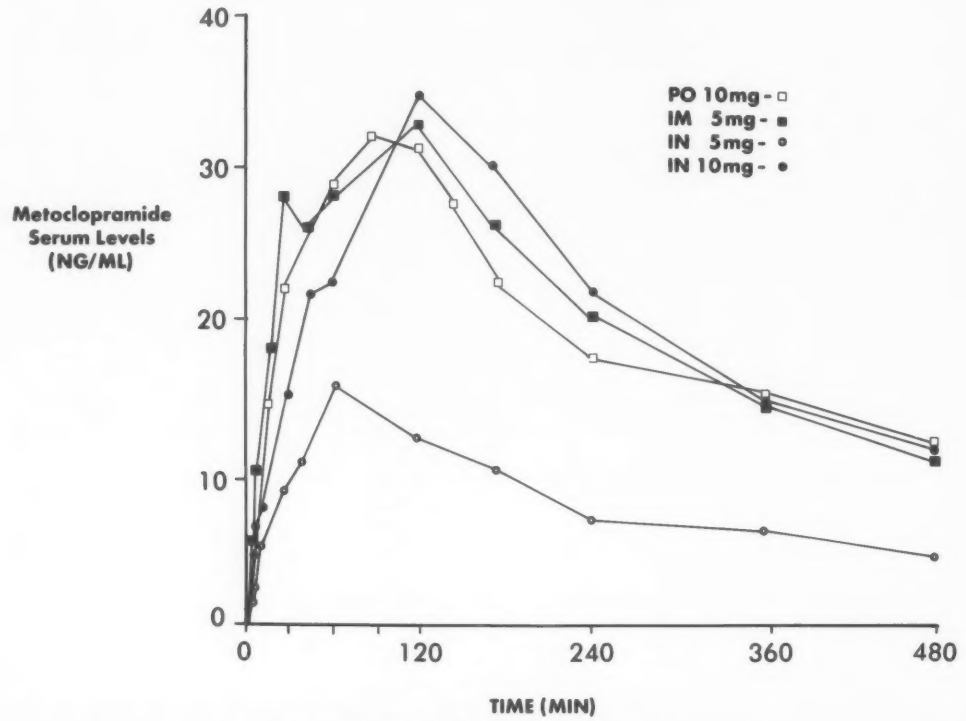


FIGURE 1.—Bioavailability and pharmacokinetics of metoclopramide.

IM, and 10-mg IN doses. The 10-mg IN doses resulted in an average C_{max} value 2% less than C_{max} values observed following PO administration and 3% greater than those observed following IM administration. The average C_{max} value following the 5-mg IN doses was significantly different ($P < 0.05$), being 43.3% that of the 10-mg IN doses. The average T_{max} values for the 5- and 10-mg IN doses were 80.6 and 131 minutes, respectively; these values were found to be significantly different ($P < 0.05$). None of the values for Kel and T½ were found to be statistically significantly different.

In all subjects, IN metoclopramide was well tolerated and did not cause any local side effects on the nasal mucosa.

DISCUSSION

It was observed in this study that metoclopramide follows similar absorption and elimination characteristics when administered via three different extravascular routes: IN, IM, and PO. However, comparison of specific metoclopramide plasma concentration data revealed statistically significant differences in some of the AUC, C_{max}, and T_{max} values.

The most unexpected finding in the study was that the 5-mg IM dose resulted in a mean AUC value that was similar to the 10-mg IN and 10-mg PO values. This suggests that both the IN and the PO formulations show consistently limited absorption from the extravascular site, while the IM formulation shows absorption which is two times as high as the others. First-pass metabolism in the liver is a potential explanation for the PO formulation data, but it is not likely to explain the IN data, since the nasal vasculature does not deliver blood directly to the hepatic circulation and thus avoids significant first-pass metabolism.

It is remarkable that there were few statistically significant differences between the 10-mg IN, 10-mg PO, and 5-mg IM formulations with respect to the other pharmacokinetic parameters, namely, C_{max} and T_{max}. Values observed following the 5-mg IM doses were similar to those associated with the 10-mg IN and the 10-mg PO doses. However, a statistically significant difference in T_{max} values between the 10-mg IN and the 5-mg IN formulations was noted. The results may reflect the limitations of a small study population, limited metoclopramide plasma concentration data, or a nasal membrane phenomenon which delays absorption of the 10-mg IN preparation.

Since metoclopramide plasma concentration data were collected for only 8 hours after drug administration, and absorption, distribution and elimination phases were not separated out mathematically, it is difficult to know the exact times during which elimination was the only active pharmacokinetic function. Thus, T½ values reported in this study are implicitly variable. In spite of these limitations, Kel and T½ values reported here were not statistically different.

The results of this study show consistent and relatively predictable metoclopramide plasma concentrations following IN administration of the drug. Although some pharmacokinetic differences were noted, the overall AUC data suggest that blood concentrations of metoclopramide following IN and PO administration (at equivalent doses) are comparable. A clinical inference drawn from this observation is that therapeutic effects following IN administration are likely to be similar to those following PO administration. However, delays in drug uptake from an extravascular site (ie, high T_{max}) may cause a delay in maximal therapeutic effect or reduction in pharmacologic intensity.

Currently, we are evaluating the efficacy of higher doses of IN metoclopramide for cancer-associated nausea and vomiting. Further definition of the safety and efficacy of IN metoclopramide in large clinical trials is warranted in determining potential future use of this antiemetic agent.

REFERENCES

1. GRALLA RJ, ITRI LM, PISKO SE, ET AL. Antiemetic efficacy of high-dose metoclopramide: randomized trials with placebo and prochlorperazine in patients with chemotherapy-induced nausea and vomiting. N Engl J Med 305:905-909, 1981.
2. GARNICK MB. Letter: Oral metoclopramide and cisplatin chemotherapy. Ann Intern Med 99:127, 1983.
3. RITSCHEL WA. Handbook of Basic Pharmacokinetics, 2nd ed. Hamilton, IL, Drug Intelligence Publications, Inc, 1982, pp 275-277.

Phase II Study of Etoposide in Previously Untreated Squamous Cell Carcinoma of the Head and Neck[1]

Melody A. Cobleigh,* James H. Hill, Thomas E. Lad, Daniel E. Shevrin, and Edward L. Applebaum[2]

We have evaluated etoposide as a single agent against previously untreated squamous cell cancer of the head and neck (SCC H/N).

METHODS

Fourteen patients were entered in this phase II study. Patients were registered at a central site. All registered patients are reported here (table 1). A dose of 100 mg/m^2 was administered daily for 5 days as an infusion over 45 minutes at 3-week intervals. Dosages were modified according to the wbc and platelet counts obtained on the date of treatment. If the wbc count was between 2000 and 3000/mm^3 or the platelet count was between 75,000 and 100,000/mm^3, 50% of the initial dose was administered. If the wbc count was < 2000/mm^3 or the platelet count was $< 50{,}000$/mm^3, therapy was withheld and the patient was evaluated weekly until counts returned to levels acceptable for treatment. Eligibility criteria were as follows: histologically confirmed SCC H/N; evaluation by a treatment team consisting of head and neck cancer surgeons, radiation oncologists, and medical oncologists resulting in a consensus opinion that the patient was incurable by surgery, irradiation, or combined surgery and irradiation; performance status of at least 3 by Eastern Cooperative Oncology Group (ECOG) criteria; life expectancy > 6 weeks; signed informed consent; no prior surgery, irradiation, or chemotherapy; wbc and platelet counts > 3000 and 100,000/mm^3, respectively; bilirubin and creatinine < 1.5 and 2 mg/dl, respectively; ability of the patient to cooperate with therapy; and measurable tumor.

Patients were evaluable if they received two cycles of therapy, progressed after one cycle of therapy, or died as a result of therapy. Patients were examined by a medical oncologist and a head and neck cancer surgeon prior to each treatment. Tumors which were not strictly measurable such as a nasopharyngeal primary site were evaluated endoscopically. If these tumors were clearly enlarging, patients were considered to have failed, even if measurable sites of tumor, such as regional lymph nodes, had not progressed. Tumor size was defined as the sum of the products of the perpendicular diameters of measurable indicator lesion(s) where one diameter was the maximal lesion diameter. A 25% increase in tumor size was considered progressive disease. If tumor progression did not occur after one cycle of therapy, a second was administered. If tumor size had not decreased by $\geq 50\%$ after two cycles of therapy, etoposide was discontinued. Stable disease was defined as a $< 50\%$ regression and $< 25\%$ progression in tumor size after two cycles.

RESULTS

Toxicity was minimal except in patients with a performance status of 3. Two of those three patients died during Cycle 1. No dose reductions were required. The death of Patient 4 resulted directly from drug-induced neutropenic infection. Patient 8 may have died as a result of treatment as well (1). He had severe organic heart disease, was found dead 13 days after beginning etoposide, and is not evaluable.

Eleven patients progressed or died as a result of treatment. Two patients with stable disease experienced an 11% regression rate and a 6% progression rate. Conflicting results concerning activity of etoposide versus SCC H/N have appeared (2-6). Our study is the only one performed in previously untreated patients with SCC H/N. The probability that etoposide is $> 20\%$ active is $< 10\%$, even in a highly favorable group of patients.

[1] Received Sept 10, 1986; revised Oct 17, 1986; accepted Oct 27, 1986.

[2] Department of Medicine, University of Illinois at Chicago, and Department of Otolaryngology-Head and Neck Surgery, University of Illinois Hospital and West Side Veterans Administration Center, Chicago.

* *Reprint requests to:* Melody A. Cobleigh, MD, Department of Medicine, University of Illinois, 720 N Clinical Science Bldg, PO Box 6998, Chicago, IL 60680.

TABLE 1.—Clinical patient characteristics*

Patient No.	Primary site	TNM†	Stage	EPS	Response
1‡	Hypopharynx	T2N2AM0	IV	0	S
2	Base of tongue	T4N2BM0	IV	0	P
3	Anterior tongue	T2N3AM0	IV	0	P
4	Retromolar trigone	T4N3BM0	IV	3	DD
5	False vocal cord	T2N2BM1	IV	2	P
6	Pyriform sinus	T2N3AM1	IV	0	S
7	Tonsil	T4N2BM0	IV	1	P
8	Pharynx	T4N1M0	IV	3	N
9	Base of tongue	T4N3BM0	IV	3	P
10§	Base of tongue	T3N3BM0	IV	1	P
11	Tonsil	T4N2BM0	IV	1	P
12	Base of tongue	T3N3BM0	IV	3	P
13	Soft palate	T4N3BM0	IV	1	P
14	Posterior pharynx	T4N3BM0	IV	0	P

*S = stable disease; P = disease progression; DD = drug death; N = nonevaluable; and EPS = ECOG performance status.

†Classification system of the American Joint Committee on Cancer, 1979 and 1983.

‡Patient refused surgery and irradiation.

§Patient had simultaneous non-small cell lung cancer; it also progressed on treatment.

REFERENCES

1. AISNER J, VAN ECHO DA, WHITACRE M, ET AL. A phase I trial of continuous infusion VP16-213 (etoposide). Cancer Chemother Pharmacol 7:157–160, 1982.
2. EUROPEAN ORGANIZATION FOR RESEARCH ON TREATMENT OF CANCER CLINICAL SCREENING GROUP. Epipodophyllotoxin (VP16213) in treatment of acute leukemias, hematosarcomas and solid tumours. Br Med J 3:199–202, 1973.
3. NISSEN NI, PAJAC TF, LEONE LA, ET AL. Clinical trial of VP16-213 (NSC-141540) iv twice weekly in advanced neoplastic disease. A study by the CALGB. Cancer 45:232–235, 1980.
4. FELMAN IE, GRUNBERG SM, GALA KV, ET AL. Phase II trial of etoposide (VP16) in squamous cell carcinoma of the head and neck. Proc Am Assoc Cancer Res 2:162, 1983.
5. GRUNBERG S, FELMAN IE, GALA KV, ET AL. Phase II study of etoposide (VP-16) in the treatment of advanced head and neck cancer. Am J Clin Oncol 8:393–395, 1975.
6. CRIVELLARI D, VEROSINI A, DONATELLA MM, ET AL. Phase II trial of oral VP16-213 (etoposide) in patients with advanced head and neck cancer. Tumori 71:499–500, 1985.

Phase II Study of Aclarubicin in Non-Small Cell Lung Cancer[1,2]

Jane M. Leiby,[3,*] William Hicks,[3] Ralph W. Roach,[3] and James A. Neidhart[3,4]

Aclarubicin is an anthracycline antibiotic thought to perhaps have less cardiotoxicity than doxorubicin (1). Early development in Japan suggested activity in a variety of neoplasms including lung cancer (1,2). The purpose of this study was to determine the response rate of minimally pretreated non-small cell lung cancer (NSCLC) to aclarubicin.

PATIENTS AND METHODS

Eligible patients had a histologic diagnosis of NSCLC, adequate bone marrow function (wbc count $> 3000/\mu l$ and platelet count $> 100,000/\mu l$), and adequate hepatic and renal functions (bilirubin < 3.0 mg/dl and creatinine < 2.0 mg/dl). Preference was given to patients without prior chemotherapy. The starting dose of aclarubicin (100 mg/m^2) was given as a brief iv infusion and repeated every 3 weeks. Dose adjustments were made for toxicity. A partial response was defined as a 50% reduction in tumor size lasting for 1 month.

RESULTS

Twenty-one eligible patients were entered (table 1). Three other patients were registered but not included in the analysis because they were deemed ineligible after histologic review. The 21 patients received between one and five courses of aclarubicin. Two patients died of their disease after one course, and two had stable disease after one course but refused a second course because of nausea and vomiting. There were no responses seen. Toxicity consisted of myelosuppression, nausea and vomiting, and stomatitis (table 1). Evidence of cardiotoxicity was not specifically sought. There was no clinical evidence of cardiotoxicity, but the cumulative dose of aclarubicin in these patients was low.

DISCUSSION

Two other negative studies of aclarubicin in NSCLC have been reported (4,5), with a total of 56 patients stud-

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Evaluable for response/entered	17/21
Median age in yrs (range): 60 (49–70)	
Mean Zubrod performance status (range): 1.4 (0–3)	
Tumor histology ($N = 17$)	
Adenocarcinoma	6
Squamous carcinoma	7
Large cell carcinoma	3
Unknown	1
Previous therapy ($N = 21$)	
Chemotherapy (anthracycline-containing)	6 (3)
Radiotherapy (other than brain)	12
No. of courses received ($N = 21$)	
1	6
2	11
3	1
4	2
5	1
Toxicity*	
Nausea and vomiting	
Grade 4	1
Grade 3	1
Grade 2	5
Grade 1	6
Stomatitis	
Grade 3	1
Grade 2	1
Grade 1	1
Hematologic (cells/μl)	
Leukocyte count	
Grade 4 (< 1000)	1
Grade 3 (1000–1999)	5
Grade 2 (2000–2999)	2
Grade 1 (3000–3999)	4
Platelet count	
Grade 4 ($< 25,000$)	3
Grade 3 (25,000–49,000)	1
Grade 2 (50,000–74,000)	0
Grade 1 (75,000–99,000)	0

*Most severe grade achieved, Southwest Oncology Group toxicity grading criteria.

[1] Received July 30, 1986; revised Oct 24, 1986; accepted Oct 28, 1986.
[2] Supported in part by Public Health Service grants CA-16058 and CA-09338 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.
[3] The Ohio State University Comprehensive Cancer Center, Columbus.
[4] *Present address:* University of New Mexico Cancer Center, Albuquerque.
* *Reprint requests to:* Jane M. Leiby, MD, Division of Hematology/Oncology, Department of Medicine, The Ohio State University, 10 N Doan Hall, 410 W 10th Ave, Columbus, OH 43210-1228.

ied. In those studies, 15 of 21 patients and 29 of 35 patients had been previously treated with chemotherapy. Only one partial response was seen. This study had fewer heavily pretreated patients and provides convincing evidence that aclarubicin has no significant activity in NSCLC.

REFERENCES

1. OKA S, MATHE G, and MITROU PS. Aclacinomycin A. Cancer Treat Rev 11:299, 1984.
2. FURUE H, KOMITA T, NAKOO I, ET AL. Clinical experiences with aclacinomycin-A. Recent Results Cancer Res 63:241, 1978.
3. ROACH RW, UNVERFURTH DV, MALSPEIS L, ET AL. Phase I evaluation of aclacinomycin-A. Proc ASCO 1:31, 1982.
4. CHIUTEN DF, CARR DT, DHINGRA HM, ET AL. Aclacinomycin-A (ACM-A, NSC 208734) in metastatic bronchogenic carcinoma. Proc Am Assoc Cancer Res 25:180, 1984.
5. ETTINGER DS, FINKELSTEIN DM, HARPER GR, ET AL. Phase II study of mitoxantrone, aclarubicin, and diaziquone in the treatment of non-small cell lung carcinoma: an Eastern Cooperative Oncology Group study. Cancer Treat Rep 69:1033-1034, 1985.

Phase II Trial of Esorubicin in Patients With Advanced Melanoma[1,2]

Sergio Frustaci,[3,*] Giampietro Gasparini,[3] Enzo Galligioni,[3] Umberto Tirelli,[3] Nicola Di Pietro,[4] Diana Crivellari,[3] Giovanni Lo Re,[3] and Silvio Monfardini[3]

Esorubicin is a new doxorubicin analog characterized by the lack of the hydroxyl group in position 4′ of the aminosugar (1). The derivative was 1.5–3 times more toxic and more active than doxorubicin either in vitro or in vivo (2). Moreover, an important finding was the limited or absent cardiotoxicity noted in all animal models studied (3). Phase I trials showed that doses ranging from 25 to 35 mg/m^2, given iv every 3 weeks, were appropriate for phase II studies and indicated that leukopenia was the dose-limiting toxic effect (4). Because of the marginal activity of conventional antineoplastic agents in advanced melanoma, we conducted this disease-oriented phase II trial in previously untreated metastatic or locally advanced melanoma patients.

PATIENTS AND METHODS

All consecutive patients with inoperable locally advanced or metastatic melanoma, observed at our Center from July 1984 to August 1985, were entered in the study. Patient characteristics are shown in table 1. Eligibility criteria were: measurable disease; Karnofsky performance status > 60; wbc count > 4000/mm^3; platelet count > 120,000/mm^3; normal renal, hepatic, and cardiac functions; and no previous treatment with chemotherapy.

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Entered	16
Evaluable	15
Median age in yrs (range): 64 (25–79)	
Median Karnofsky score (range): 90 (60–100)	
Sites of measurable lesions	
Skin nodules	7
Lung	6
Liver	3
Lymph node	3
Viscera	1
Primary (oropharyngeal)	1
Previous treatment	
Surgery	8
Surgery + radiotherapy	6
Surgery + immunotherapy	1
None*	1

*Inoperable lesion of the oropharynx.

Esorubicin was administered iv every 3 weeks until progressive toxicity. The initial dose was 30 mg/m^2; subsequent doses were increased to 35 mg/m^2 if no hematologic toxicity was encountered 3 weeks after treatment; otherwise, drug administration was postponed by 1 week. Basal cardiological evaluation included: clinical evaluation, electrocardiogram, and echocardiography every three cycles. World Health Organization criteria for response and toxicity were employed.

RESULTS AND DISCUSSION

Sixteen patients were entered in the study. One patient was not evaluable for response because the second cycle was delayed for > 2 weeks. No other concomitant treatments were given to the evaluable patients. Patients received a median of two cycles (range, two to five).

Six patients received a dose escalation to 35 mg/m^2, whereas one patient required a dose reduction because of leukopenia. No major objective therapeutic responses were seen; two patients had no change in disease parameter (inguinal lymph nodes and primary lesion of the nasal fossa) for 2 and 4 months, respectively. Leukopenia was the most important toxic effect and was noted in 11 patients (69%). The median wbc count nadir was 2600/mm^3 (range, 600–6700). Thrombocytopenia was never observed. Grades 1 and 2 anemia were noted in three and one patients, respectively, whereas gastrointestinal toxicity and hair loss (grade 1 or 2) were observed in 62% and 56% of the patients, respectively.

[1] Received Apr 21, 1986; revised Aug 18, 1986; accepted Oct 6, 1986.
[2] Supported in part by a grant of Farmitalia Carlo Erba.
[3] Division of Medical Oncology, Centro di Riferimento Oncologico, Aviano (Pordenone), Italy.
[4] Farmitalia Carlo Erba, Milano, Italy.
* *Reprint requests to:* Sergio Frustaci, MD, Division of Medical Oncology, Centro di Riferimento Oncologico, Via Pedemontana Occ, 33081 Aviano (Pordenone), Italy.

After a median total dose of 100 mg/m^2 (range, 90–170), follow-up cardiac evaluation did not show any cardiotoxicity in five patients. This phase II trial was conducted in previously untreated patients with good clinical conditions and well-measurable lesions. In fact, superficial lymph nodes or skin nodules represent the majority of disease parameters. Nevertheless, objective tumor regressions were not observed. Therefore, the real response rate of the drug is $< 20\%$ with 0.95 probability.

Our clinical results are in agreement with a large in vitro study performed in Milan in which esorubicin revealed some activity in 16% of human melanoma specimens (5). Leukopenia was the most severe acute side effect and median nadir wbc count and range indicate that an adequate dose was administered.

We conclude that in advanced melanoma, esorubicin, at the dose and schedule administered, has no activity and therefore further evaluation in this disease does not seem indicated.

REFERENCES

1. ARCAMONE F. Antitumor anthracyclines: recent developments. Med Res Rev 4:153–188, 1984.
2. CASAZZA AM, SAVI G, PRATESI G, ET AL. Antitumor activity in mice of 4′-deoxydoxorubicin in comparison with doxorubicin. Eur J Cancer Clin Oncol 19:411–418, 1983.
3. VILLANI F, COMAZZI R, GENITONI V, ET AL. Preliminary evaluation of myocardial toxicity of 4′-deoxydoxorubicin: experimental and clinical results. Drugs Exp Clin Res 3:223–231, 1985.
4. STANTON GF, RAYMOND V, WITTES RE, ET AL. Phase I and clinical pharmacological evaluation of 4′-deoxydoxorubicin in patients with advanced cancer. Cancer Res 45:1862–1868, 1985.
5. ZAFFARONI N, SILVESTRINI R, DAIDONE MG, ET AL. Comparative activity of different anthracyclines as measured by an in vitro antimetabolic assay. Proc ASCO 4:125, 1985.

Treatment of Recurrent or Metastatic Poorly Differentiated Adenocarcinoma of the Endometrium With Tamoxifen and Medroxyprogesterone Acetate[1,2]

Richard C. Kline, Ralph S. Freedman,* Lovell A. Jones, and E. Neely Atkinson[3]

TABLE 1.—Patient characteristics and responses to therapy

	Prior therapy*			Treatment with tamoxifen/MPA					
Age (yrs)	Surgery	Irradiation/ chemotherapy	Tumor sites†	No. of courses	Response‡	Duration (mos)	Status§	Survival (mos)	Comments‡¶
49	H & O	—	A, L, P	36	CR	41	A	60	—
69	H & O	ICR	A	3	NC	3	D	17	PAC (NR)**
46	H & O	PRT	P	3	NC	3	D	9	Nausea and vomiting on MPA
74	H & O	PRT	Inguinal nodes, L	4	NC	4	D	15	ACFM (PR)**
73	Biopsy	Chemotherapy	A, L	3	NC	3	D	3	—
78	Biopsy	ICR	Inguinal nodes	5	NC	4	D	10	—
76	H & O	PRT	L, P	4	NC	4	D	13	PAC (NC)**
61	H & O	—	P	1	NR	—	D	1	—
59	Biopsy	PRT	A, L	4	NC	4	D	5.5	ERP(+) PRP(+)
61	H & O	PRT	P	4	NC	4	D	19	PAC (PR)**
73	H & O	PRT/chemotherapy	A, supraclavicular nodes	4	NR	—	D	5	PAC (NR)**
58	H & O	PRT	A	5	NC	5	D	17	ERP(−) PRP(−) PAC (PR)**
65	H & O	PRT, ICR	P	2	NR	—	A	17	Megestrol (NR)**
68	H & O	ICR	A	2	NR	—	D	6	Bilateral pedal edema; PAC (NR)**
60	H & O	PRT, ICR	L	2	NR	—	D	16	AC (PR)**
70	H & O	PRT, ICR	Inguinal and supraclavicular nodes	1	NR	—	D	1.5	ERP(+) PRP(−)
63	H & O	PRT, ICR	A	15	NC	15	A	15	—
56	H & O	ICR	Lumbar spine	1	NR	—	D	6	—
77	H & O	ICR	L	2	NR	—	D	2	—
64	Biopsy	PRT, ICR	P	< 1	NR	—	D	6.5	—

*H & O = total abdominal hysterectomy and bilateral salpingo-oophorectomy; ICR = intracavitary radium; and PRT = pelvic irradiation.
†A = abdomen; L = lungs; and P = pelvis.
‡CR = complete response; NC = no change; NR = no response; and PR = partial response.
§A = alive; and D = dead.
¶PAC = cisplatin, doxorubicin, and cyclophosphamide; ACFM = doxorubicin, cyclophosphamide, 5-fluorouracil, and MPA; ERP = estrogen receptor protein; PRP = progesterone receptor protein; ( ) = response; and AC = doxorubicin and cyclophosphamide.
**Indicates subsequent therapy.

[1] Received May 6, 1986; revised Sept 23, 1986; accepted Oct 6, 1986.
[2] Supported in part by Public Health Service grant CA-31382 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. R. C. Kline is an American Cancer Society Junior Fellow.
[3] Departments of Gynecology and Biomathematics, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.
* *Reprint requests to:* Ralph S. Freedman, MD, PhD, Department of Gynecology, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, Houston, TX 77030.

Recurrent or metastatic poorly differentiated endometrial carcinoma responds to progestogens alone in only about 10% of patients (1). A relative or absolute deficiency of progesterone receptor protein (PRP) could account for the low response rate (2). Tamoxifen has been shown to increase PRP values in endometrial cancer tissues (3,4), suggesting that therapeutic synergism might result from the sequential administration of tamoxifen plus a progestogen.

Twenty consecutive patients with recurrent or metastatic poorly differentiated endometrial carcinoma received sequentially administered courses of tamoxifen and medroxyprogesterone acetate (MPA). Treatment consisted of tamoxifen (10 mg) twice a day for 5 days followed by MPA (50 mg) twice a day on Days 6–25. The cycle was recommenced after a 5-day rest period.

Patient characteristics are shown in table 1. Patient ages ranged from 46 to 78 years (median, 64). Sixteen patients had undergone a total abdominal hysterectomy and bilateral salpingo-oophorectomy and four had had biopsy alone; of those patients, 17 had received irradiation as initial therapy. Two patients had previously received chemotherapy, but none had received hormones in the treatment of their cancers. Fresh tissue was available for receptor analysis from three patients.

One patient with pulmonary metastasis and surgically confirmed extrauterine pelvic disease had a complete response to therapy. Ten patients had stable disease for ≥ 8 weeks from treatment start, with a median duration of 4 months (range, 3–15). Three patients are alive at 15, 17, and 60 months. Four of nine patients responded to other subsequent therapy. There was no significant toxicity in the group. Symptoms included nausea and vomiting, presumed to be due to MPA, in one patient and bilateral pedal edema in another.

Although previous investigators have reported an increase in PRP content in primary endometrial carcinomas consequent to a short course of tamoxifen (3,5), we are unable to conclude that the sequential administration of tamoxifen and MPA at the dosage and schedule that we used has a significant effect in recurrent endometrial cancer originating from grade 3 or poorly differentiated tumors. It is possible that a better response rate may be achieved by administering higher doses of tamoxifen or MPA or by substituting for tamoxifen a different PRP inducer such as ethinyl estradiol.

REFERENCES

1. KOHORN EI. Gestagens and endometrial carcinoma. Gynecol Oncol 4:398–411, 1986.
2. EHRLICH CE, YOUNG PCM, and CLEARY RE. Cytoplasmic progesterone and estradiol receptors in normal, hyperplastic, and carcinomatous endometria: therapeutic implications. Am J Obstet Gynecol 141:539–546, 1981.
3. ROBEL P, GRAVANIS A, ROGER-JALLAIS L, ET AL. Female sex steroid receptors in postmenopausal endometrial carcinoma. Biochemical responses to anti-estrogen and progestin. Prog Clin Biol Res 142:167–179, 1984.
4. SCHWARTZ PE, MACLUSKY N, NAFTOLIN F, ET AL. Tamoxifen-induced increase in cytosol progestin receptor levels in a case of metastatic endometrial cancer. Gynecol Oncol 16:41–48, 1983.
5. CARLSON JA, JR, ALLEGRA JC, DAY TG, JR, ET AL. Tamoxifen and endometrial carcinoma: alterations in estrogen and progesterone receptors in untreated patients and combination hormonal therapy in advanced neoplasia. Am J Obstet Gynecol 149:149–153, 1984.

Phase II Trial of Cisplatin and Etoposide in Adenocarcinomas of the Upper Gastrointestinal Tract[1,2]

D. P. Kelsen,* J. Buckner, A. Einzig, G. Magill, R. Heelan, and V. Vinciguerra[3]

Several recent phase II trials have indicated that cisplatin as a single agent has 10%–20% activity in gastric cancer (1–3). A phase II trial of the epipodophyllotoxin etoposide (VP-16-213) demonstrated a 12% response rate in previously untreated patients (4). Because these two agents appear to have a synergistic antineoplastic effect both in vitro and in some clinical trials, a phase II trial of cisplatin plus VP-16-213 was performed in patients with advanced adenocarcinomas of the upper gastrointestinal tract (5,6).

MATERIALS AND METHODS

Thirty-six patients with advanced adenocarcinomas of the upper gastrointestinal tract were entered in this study. All patients had measurable or evaluable disease and fulfilled the following eligibility requirements: advanced tumor not amenable to potentially curative therapy; adequate bone marrow reserve; Karnofsky performance status ≥ 60; creatinine clearance ≥ 50 ml/minute/1.73 m^2; clinically normal auditory function; and ability to give informed consent.

The treatment plan included cisplatin (60 mg/m^2) given on Days 1 and 29, and every 6 weeks thereafter, and VP-16-213 (100 mg/m^2) given in three divided doses (total dose, 300 mg/m^2) during the 1st and 5th weeks (Days 3, 5, 7 and 31, 33, 35, respectively). Following the first two courses, VP-16-213 was given every 3 weeks, again in three divided doses. Thus, following the first month of therapy, cisplatin plus VP-16-213 was alternated with VP-16-213 alone. One cycle of cisplatin plus VP-16-213 was considered the minimum requirement for response assessment.

RESULTS

Of the 36 patients entered in the study, 33 were evaluable for response. All patients were evaluable for toxicity.

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Entered	36
Evaluable	33
Median age in yrs (range): 62 (31–76)	
Median Karnofsky performance status (range): 80 (60–90)	
Males:females	26:10
Prior therapy	
None	24
Chemotherapy	8
Radiation therapy	1
Chemotherapy and radiation therapy	3
Location of primary	
Esophagus	6
GE junction	8
Gastric	22
Response	
Complete	0
Partial	1

Patient characteristics are shown in table 1. Sites of metastatic disease included lymph nodes (six), lung (five), liver (16), and primary (13). Many patients had more than one measurable site. Ten patients had the esophageal or gastric lesion as the only site of measurable disease.

Only one major objective regression was noted (3%, 95% confidence limits, 0%–9%); this patient had a GE junction primary and had not received prior chemotherapy; his response lasted for 3 months.

Toxicity was, in general, tolerable. The median peak creatinine was 1.2 mg/100 ml. Myelosuppression was moderate, with a median wbc count nadir of 2500/mm^3

[1] Received June 9, 1986; revised Sept 22, 1986; accepted Oct 6, 1986.

[2] Supported in part by contract N01-CM-07337 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] Solid Tumor Service, Departments of Medicine and Diagnostic Radiology, Memorial Sloan-Kettering Cancer Center; Cornell University Medical College, New York; and the Community Clinical Oncology Program, Manhasset, NY.

* *Reprint requests to:* David Kelsen, MD, Solid Tumor Service, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021.

(range, 200–12,600); the median platelet count nadir was 149,000/mm^3 (range, 17,000–336,000).

Other toxic effects included alopecia and nausea and vomiting (both of which were common). Two patients had a mild peripheral neuropathy.

DISCUSSION

A variety of chemotherapy combinations, most based on 5-fluorouracil (5-FU), have been studied as palliative treatment for patients with advanced gastric cancer. Although there is no clear evidence that any one regimen is markedly superior to another, the most widely used combination, FAM (5-FU, doxorubicin, and mitomycin), has been reported to induce major objective responses in 25%–55% of patients. More controversial has been the effect of FAM therapy on survival duration. A recent study by Cullinan et al (8) suggested that there was no significant survival advantage for FAM when compared to either 5-FU and doxorubicin or to 5-FU alone. This trial supports the use of investigational therapy in previously untreated patients in an attempt to develop new regimens in this disease.

The low response rate seen with cisplatin and VP-16-213 in the current study was disappointing. Although dosage escalation to moderate myelosuppression was not uniformly performed, a number of patients had significant leukopenia or thrombocytopenia without response.

We conclude that, using these dosages and schedules, cisplatin and VP-16-213 has only modest activity in adenocarcinomas of the upper gastrointestinal tract. We do not plan further study of this combination in this disease.

REFERENCES

1. LEICHMANN L, MCDONALD B, DINDOGRU A, ET AL. Cisplatin: an active drug in the treatment of disseminated gastric cancer. Cancer 53:18–22, 1984.
2. AABO K, PEDERSEN H, and RØRTH M. Cisplatin in the treatment of advanced gastric carcinoma: a phase II study. Cancer Treat Rep 69:449–450, 1985.
3. LACAVE A, IZARZUGAZA I, ANTÓN APARICIO LM, ET AL. Phase II clinical trial of *cis*-diamminedichloroplatinum(II) in gastric cancer. Am J Clin Oncol 6:35–38, 1983.
4. KELSEN DP, MAGILL GB, CHENG E, ET AL. Phase II trial of etoposide in adenocarcinomas of the upper gastrointestinal tract. Cancer Treat Rep 67:509–510, 1983.
5. SCHABEL FM, JR, TRADER MW, LASTER WR, JR, ET AL. *cis*-Dichlorodiammineplatinum(II): combination chemotherapy and cross-resistance studies with tumors in mice. Cancer Treat Rep 63:1459–1473, 1979.
6. WILLIAMS S, EINHORN LH, GRECO FA, ET AL. VP-16-213 salvage therapy for refractory germ cell neoplasms. Cancer 46:2154–2158, 1980.
7. KELSEN DP, HEELAN R, and COONLEY C. Clinical and pathological criteria of response to chemotherapy in patients with esophageal cancer. Am J Clin Oncol 6:539–546, 1983.
8. CULLINAN S, MOERTEL C, FLEMING T, ET AL. A comparison of three chemotherapeutic regimens in the treatment of advanced pancreatic and gastric carcinoma. JAMA 253:2061–2067, 1985.

Phase II Studies of PCNU and Bisantrene in Advanced Renal Cell Carcinoma[1,2]

Paul J. Elson,* Robert H. Earhart, Larry K. Kvols, Robert Spiegel, Alan M. Keller, Merrill S. Kies, Thomas E. Davis, Christine Stevens, Lora Gumas, and Donald L. Trump[2]

This report summarizes the latest in a series of phase II clinical trials, conducted by the Eastern Cooperative Oncology Group (ECOG), in patients with advanced renal cell carcinoma. PCNU, a new nitrosourea with high alkylating activity, and bisantrene, a new anthracene compound, were evaluated for efficacy and toxicity in separate, nonrandomized trials between February and December 1981.

PATIENTS AND METHODS

Patients were eligible for these trials if they had measurable metastatic or recurrent renal adenocarcinoma not amenable to surgery; ECOG performance status < 4 (1); no chemotherapy or radiotherapy within the previous month; no major surgical procedures within the previous 3 weeks; no prior treatment with a study drug; adequate bone marrow [total wbc count $\geq 4000/mm^3$, platelet count $\geq 100,000/mm^3$ (PCNU) or $\geq 130,000/mm^3$ (bisantrene)], renal (serum creatinine < 1.6 mg/dl), and hepatic (direct bilirubin ≤ 1.5 mg/dl) functions; and signed informed consent. Entry in the bisantrene trial was further restricted to patients with no significant heart disease and limited, or no, exposure to doxorubicin (cumulative dose < 300 mg/m^2) and radiotherapy (biologic equivalent < 2500 rads in 13 fractions).

PCNU was administered at a dose of 100 mg/m^2 iv over 30 minutes in good-risk patients and 75 mg/m^2 in poor-risk patients every 6 weeks (level of risk was determined by previous treatment with other nitrosoureas and extent of prior radiotherapy). Patients treated with bisantrene received 260 mg/m^2 iv in 500 ml of 5% dextrose in water over 90 minutes every 4 weeks. Standard ECOG criteria (1) were used to evaluate response and toxicity. Treatment was continued until disease progression or unacceptable toxicity occurred.

RESULTS AND DISCUSSION

A total of 80 patients were entered in the two trials (PCNU, 47 patients; bisantrene, 33). Two patients treated with PCNU and four treated with bisantrene were excluded from all analyses (never received treatment, three patients; inadequate bone marrow or renal function, three). Characteristics of the 74 evaluable patients are shown in table 1.

There were no objective responses reported among the 45 evaluable patients treated with PCNU (exact 95% confidence interval, 0%–8%). Median survival for these patients was 5.1 months. One complete and two partial responses were reported among the patients treated with bisantrene (exact 95% confidence interval, 2%–27%). The responses were not durable, however, lasting only 2, 2, and 6 months, respectively. All three responses occurred in previously untreated patients. Median survival for the bisantrene-treated patients was 4.6 months.

Myelosuppression was the major toxic effect reported by patients treated with PCNU. Fourteen patients (31%) experienced severe or worse thrombocytopenia, leukopenia, and/or anemia (table 2). Other toxic effects re-

[1] Received Sept 16, 1985; revised Aug 26, 1986; accepted Oct 6, 1986.

[2] This study was conducted by the Eastern Cooperative Oncology Group (*Paul P. Carbone*, chairman, CA-21115) and was supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The following members participated in the study: Dana-Farber Cancer Institute, Boston, MA (*P. J. Elson, C. Stevens,* and *L. Gumas*, CA-23318); Wisconsin Clinical Cancer Center, Madison (*R. H. Earhart, T. E. Davis,* and *D. L. Trump*, CA- 21076); Mayo Clinic, Rochester, MN (*L. K. Kvols*, CA-13650); New York University Medical Center, New York (*R. Spiegel*, CA-16395); Natalie Warren Bryant Cancer Center, Tulsa, OK (*A. M. Keller*); and Northwestern University Medical Center, Chicago, IL (*M. S. Kies*, CA-17145). Other participating institutions include: Albany Medical College, NY (CA-06594); Albert Einstein College of Medicine, Bronx, NY (CA-14958); American Oncologic Hospital, Philadelphia, PA (CA-18281); Chicago Medical School, IL (CA-14144); Hahnemann Medical College, Philadelphia, PA (CA-13611); Johns Hopkins Oncology Center, Baltimore, MD (CA-16116); Medical College of Ohio, Toledo; University of Minnesota, Minneapolis (CA-20365); University of Ottawa, Ontario, Canada; Hospital of the University of Pennsylvania, Philadelphia (CA-15488); University of Rochester Cancer Center, NY (CA-11083); Roswell Park Memorial Institute, Buffalo, NY (CA-12296); Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL (CA-25988); University of Pretoria, South Africa (CA-21692); Swiss Group, Bern, Switzerland; Tufts University, Walpole, MA (CA-07190); and Yale School of Medicine, New Haven, CT (CA-15485).

* *Reprint requests to:* Paul J. Elson, MS, Division of Biostatistics and Epidemiology, Dana-Farber Cancer Institute, 44 Binney St, Boston, MA 02115.

TABLE 1.—Patient characteristics*

Characteristic	PCNU	Bisantrene
Sex		
Male	24 (53)	16 (55)
Female	21 (47)	13 (45)
Age (yrs)		
Median	58	58
Range	21-77	37-75
Initial performance status		
Ambulatory (ECOG 0,1)	29 (64)	26 (90)
Nonambulatory (ECOG 2,3)	16 (36)	3 (10)
Prior chemotherapy		
None	29 (64)	17 (59)
Hormonal	4 (9)	2 (7)
Cytotoxic	4 (9)	6 (21)
Cytotoxic and hormonal	8 (18)	4 (14)
Prior nephrectomy		
No	12 (27)	8 (28)
Yes	33 (73)	21 (72)
Prior radiotherapy		
No	21 (47)	21 (72)
Yes	24 (52)	8 (28)
No. of cycles of chemotherapy		
Mean	2.1	2.3
Median	2	2
Range	1-6	1-7
Response and survival		
Complete	0	1 (3)
Partial	0	2 (7)
No response	45 (100)	26 (90)
Median survival (mos)	5.1	4.6

*Unless otherwise specified, values = No. of patients (%).

ported include mild (22 patients) to severe (two) nausea, diarrhea (five), and one patient each with dry skin, dizziness, shortness of breath, constipation, edema, and a transient elevation in BUN. The principal toxic effects reported by the patients treated with bisantrene were local toxic effects, such as phlebitis and pain, at the site of infusion (table 2). Thirty-four percent of the patients (ten of 29) given bisantrene experienced such reactions.

TABLE 2.—Toxicity

Toxicity	No. of patients (%)
PCNU	
Thrombocytopenia (cells/mm^3)	
25,000–< 50,000	6 (13)
< 25,000	3 (7)
Leukopenia (cells/mm^3)*	
1000–< 2000	1 (2)
Anemia*	
Required transfusions	5 (11)
Bisantrene	
Local reactions	
Phlebitis	7 (24)
Skin rash	2 (7)
Pain	1 (3)

*1 patient experienced both severe leukopenia and severe anemia.

Other reported toxic effects include moderate (five patients) to severe (one) leukopenia, anemia requiring transfusions (one), mild neuropathies (five), and transient elevations in serum creatinine (four).

The results of the present studies are consistent with other phase II trials of PCNU (2) and bisantrene (3,4) and indicate that neither PCNU nor bisantrene has significant activity in the treatment of advanced renal cell carcinoma.

REFERENCES

1. OKEN MM, CREECH RH, TORMEY DC, ET AL. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.
2. HARVEY JH, SMITH FP, BOWERS MW, ET AL. Phase II trial of PCNU in advanced renal cell carcinoma and malignant melanoma. Cancer Treat Rep 68:1049-1050, 1984.
3. MYERS JW, VON HOFF DD, COLTMAN CA, JR, ET AL. Phase II evaluation of bisantrene in patients with renal cell carcinoma. Cancer Treat Rep 66:1869-1871, 1982.
4. EVANS WK, SHEPHERD FA, BLACKSTEIN ME, ET AL. Phase II evaluation of bisantrene in patients with advanced renal cell carcinoma. Cancer Treat Rep 69:727-728, 1985.

Phase II Clinical Trial of Tricyclic Nucleoside Phosphate for Advanced Colorectal Cancer[1,2]

Michael J. O'Connell,* Joseph Rubin, Richard G. Hahn, Larry K. Kvols, and Charles G. Moertel[3]

Tricyclic nucleoside 5′-phosphate (TCN-P) is a water-soluble phosphate ester of tricyclic nucleoside. TCN-P has been shown to inhibit DNA, RNA, and protein synthesis, and to have antitumor activity in a variety of animal tumor systems and against a human mammary xenograft (1–3). The pharmacokinetics of this agent in humans have been described (4). Phase I studies identified hepatotoxicity as the dose-limiting toxicity, with thrombocytopenia, hyperglycemia, vomiting, and hypocalcemia also observed in some patients (5).

MATERIALS AND METHODS

All patients had histologically documented metastatic colorectal adenocarcinoma. Each patient had a measurable indicator lesion, pretreatment SGOT and alkaline phosphatase within twice the upper limit of normal, and direct-reacting bilirubin, wbc count, platelet count, and serum creatinine within normal limits. Fasting blood sugar was $\leq$ 120 mg/dl and serum calcium was $\geq$ 8.5 mg/dl.

TCN-P was given iv over 15 minutes in 50 ml of 5% dextrose in water. The initial dose was 250 mg/m^2, with a planned reduction of subsequent doses to 165 mg/m^2. Treatment was repeated at 3-week intervals.

Decrease in the size of the primary indicator lesion by $\geq$ 50%, or a $\geq$ 30% decrease in linear measurements of the liver was required to document an objective tumor response (table 1).

TABLE 1.—Patient characteristics and results

	No. of patients
Clinical parameter	
Sex (male/female)	18/13
Performance status* (0-1/2-3)	24/7
Prior chemotherapy (no/yes)	15/16
Primary indicator lesion	
Lung/liver	13/10
Lymph nodes/abdominal mass	4/3
Skin	1
Treatment effect	
Objective tumor response	1 (3%)
Median time to disease progression (wks): 6†	
Median survival time (wks): 18†	

*Eastern Cooperative Oncology Group score [0 (fully active) to 4 (totally disabled)].

†Measured from start of TCN-P therapy.

RESULTS

The median age was 59 years (range, 27–77). The lower proportion of hepatic indicator lesions than is seen in most advanced colorectal cancer studies may be explained by the pretreatment requirement for near normal liver function.

A single patient experienced partial regression of a palpable abdominal mass which lasted 6 weeks. The 95% confidence interval for objective response rate was 0%–17%.

No life-threatening or lethal toxic effects from TCN-P were observed. Six patients (19%) had an increase in fasting blood sugar (maximum value 167 mg/dl), but only one of these patients required treatment with oral hypoglycemics. There were no cases of hepatic toxicity (table 2).

TABLE 2.—Toxic effects in 31 patients

Toxic effect	% of patients
Vomiting (severe)	16
Thrombocytopenia	13
Leukopenia	6
Hyperglycemia	19
Hypocalcemia (asymptomatic)	16
Hepatic toxicity	0

[1] Received Aug 5, 1986; revised Nov 3, 1986; accepted Nov 7, 1986.

[2] Supported by contract N01-CM-57733 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3] Division of Medical Oncology, Mayo Clinic, Rochester, MN.

* *Reprint requests to:* Michael J. O'Connell, MD, Department of Oncology, Mayo Clinic, Rochester, MN 55905.

DISCUSSION

This trial has documented the lack of clinically useful activity of TCN-P in the treatment of patients with advanced colorectal cancer with the dose and schedule employed. Toxicity was tolerable using this intermittent treatment regimen and, in particular, there were no cases of hepatic toxicity.

REFERENCES

1. CLINICAL BROCHURE. Tricyclic nucleoside-5′-phosphate (NSC-280594). Bethesda, MD, National Cancer Institute, 1982.
2. ROTI ROTI LW. 5′-Phosphate of tricyclic nucleoside: metabolism and cytotoxicity in L1210 cell cultures. Proc Am Assoc Cancer Res and ASCO 18:219, 1977.
3. COBB WR, BOGDEN AE, REICH SD, ET AL. Activity of two phase I drugs, homoharringtonine and tricyclic nucleotide, against surgical explants of human tumors in the 6-day subrenal capsule assay. Cancer Treat Rep 67:173–178, 1983.
4. POWIS G, BASSECHES PJ, KROSCHEL DM, ET AL. Disposition of tricyclic nucleoside-5′-monophosphate in blood and plasma of patients during phase I and II clinical trials. Cancer Treat Rep 70:359–362, 1986.
5. MITTELMAN A, CASPER ES, GODWIN TA, ET AL. Phase I study of tricyclic nucleoside phosphate. Cancer Treat Rep 67:159–162, 1983.

Phase II Study of Vinblastine and Bleomycin in Advanced Ovarian Cancer[1]

R. J. Osborne,[2,*] V. J. Harvey,[3] M. L. Slevin,[2] J. H. Shepherd,[4] and C. J. Williams[5]

For patients with ovarian cancer refractory to first-line chemotherapy, further treatment options are bleak (1) and active second-line agents are required. Vinblastine and bleomycin have both demonstrated activity against ovarian cancer in vitro (2,3), and we have therefore investigated the use of these two drugs in combination in patients with advanced refractory ovarian cancer.

PATIENTS, METHODS, AND RESULTS

Nineteen patients with stage III or IV ovarian carcinoma were treated. One patient had received no prior treatment (having refused cisplatin-based chemotherapy). The remainder were refractory to conventional therapy. All patients had disease which was evaluable or measurable by clinical and/or imaging means. Patient characteristics are shown in table 1.

Treatment consisted of bleomycin [15 mg weekly (sc, iv, or ip)] and vinblastine (6 mg/m^2 iv) administered either weekly (to nine patients treated at the Southampton Center) or every 3 weeks (to ten patients treated at the London Center).

Patients were evaluable for response at 3-week intervals employing clinical examination and, where indicated, imaging assessment. Response was evaluated using World Health Organization (WHO) criteria (4), and treatment was continued until disease progression.

No complete or partial remissions were observed in the 17 patients evaluable for response (two patients died before completing one course of treatment). One patient who had previously been treated with chlorambucil, followed by cisplatin, doxorubicin, and cyclophosphamide, followed by ifosfamide, had stable disease during 8 months of treatment with vinblastine and bleomycin (as assessed by radiologic examination of pulmonary metastases) before progressing while on treatment. Median survival from start of vinblastine and bleomycin was 2 months (range, 0.5–9.5).

Toxicity was generally mild (table 2) with the exception of grade 3 thrombocytopenia in one patient and grade 4 leukopenia in one patient, both previously extensively treated. Constipation with abdominal pain was a major problem, occurring in most patients (with no apparent difference between the weekly or 3-week regimens). One patient treated with eight courses of bleomycin (total dose, 360 mg) developed abnormal pul-

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Entered	19
Evaluable	17
Median Karnofsky status (range): 60% (50%–80%)	
Mean age in yrs (range): 58.8 (39–77)	
Stage	
III	12
IV	7
Histology	
Serous	13
Endometrioid	4
Undifferentiated	2
Grade	
Moderately differentiated	9
Poorly differentiated	10
Disease extent	
Bulky	18
Nonbulky	1*
Prior chemotherapy	
None	1
1 regimen	8
2 regimens	8
3 regimens	2
Response	
Complete and partial	0
Stable disease	1†
Disease progression	16

*Pleural disease.
†8 mos.

[1] Received July 19, 1985; revised Oct 27, 1986; accepted Nov 17, 1986.
[2] Imperial Cancer Research Fund, Department of Medical Oncology, St. Bartholomew's and Homerton Hospitals, London, England.
[3] Department of Clinical Oncology, Auckland Hospital, New Zealand.
[4] Department of Gynaecology, St. Bartholomew's Hospital, London, England.
[5] CRC Department of Medical Oncology, Southampton General Hospital, Southampton, England.
Reprint requests to: Dr. R. J. Osborne, Imperial Cancer Research Fund, Department of Medical Oncology, St. Bartholomew's Hospital, London EC1A 7BE, England.

TABLE 2.—Toxicity*

	Worst WHO grade				
	0	1	2	3	4
Fever	12	0	4	1	0
Nausea and vomiting	11	1	3	2	0
Hemoglobin	15	0	2	0	0
Leukopenia	13	2	1	0	1
Thrombocytopenia	15	1	0	1	0

*Values = No. of patients.

monary function tests, but also had progressive intrapulmonary tumor.

The lack of activity demonstrated in this study by vinblastine and bleomycin in patients with previously treated ovarian cancer is disappointing in view of the positive findings from human tumor cloning assay studies employing tissue from refractory patients. Prospective, correlative studies based on the human tumor cloning assay are required, and any future studies employing vinblastine and bleomycin should preferably be performed in patients who have received less prior treatment.

REFERENCES

1. STANHOPE CR, SMITH JP, and RUTLEDGE F. Second trial drugs in ovarian cancer. Gynecol Oncol 5:52–58, 1977.
2. ALBERTS DS, CHEN HSG, SOEHNLEN B, ET AL. In vitro clonogenic assay for predicting response of ovarian cancer to chemotherapy. Lancet i:340–342, 1980.
3. WELANDER CE, HOMESLEY HD, and JOBSON VW. In vitro chemotherapy testing of gynecologic tumors. Basis for planning therapy? Am J Obstet Gynecol 147:188–195, 1983.
4. MILLER AB, HOOGSTRATEN B, STAQUET M, ET AL. Reporting results of cancer treatment. Cancer 47:207–214, 1981.

BOOK REVIEW

Principles and Management of Testicular Cancer. **N. Javadpour, Editor. New York, Thieme Inc., 1986, 413 pp, ISBN 0-86577-194-4**

This 413-page, 31-chapter book contains some superb individual chapters and accurately represents the knowledge base relevant to testicular cancer research. The chapters cover the entire range of information relevant to testicular cancer, the anatomy and embryology of the testicle, surface antigen analysis of normal and malignant germ cells by Solter and Knowles, a comprehensive review of risk factors and epidemiology of testicular cancer (a particularly excellent chapter by Potter and Goedert), an excellent review of carcinoma in situ by Skakkebaek and coauthors, and an outstanding review of the management of disseminated testicular cancer by Torti and Lum. In addition, there is an excellent review of the history of testicular cancer research by Blandy and Oliver. The chapter on infertility in patients with testicular cancer and the chapter on testicular tumors in children were also particularly well done and worthwhile.

Unfortunately, these gems are marred by repetitious and redundant chapters which fill the book. The introductory chapter has many spelling and editorial inconsistencies and mistakes. For example, on two occasions the reader is told that "randomized control [sic] trials" are necessary. We are also told that "double dose of platinum [sic] is the treatment of choice for stage III testis cancer with poor prognostic features." And lastly we are told that "the Danish Cancer Society has collaborated an Intergroup study," a factual and grammatical error. There are also several minor but irritating factual errors throughout other chapters. A chapter entitled "Clinical Features and Natural History of Testicular Cancer" adds nothing to the value of the book, and an entire chapter devoted to the technique of radical orchiectomy is superfluous. Additionally, there are four chapters on radiologic and staging questions. The chapter on serum tumor markers is simplistic and did not reflect an adequate review of the literature. Lastly, the chapter on bulky disseminated non-seminoma testicular cancer with poor prognostic features seems to have been gerrymandered into the book and contributes very little to the knowledge base.

The book displays a relative evenhandedness in its review of the literature. Most references are dated between 1980 and 1984, although there are none after 1984. There is a relative paucity of references to the important consecutive studies produced at Indiana University and the Southeastern Cancer Study Group by Dr. Einhorn and associates. Obvious personal biases have crept into some chapters, such as the arbitrary classification schema for patients with poor-prognosis metastatic testicular cancer. That schema, offered by the editor, has not been supported by careful multivariate analysis of large populations of such patients.

In conclusion, the book offers a broad and yet in-depth review of many areas of testicular cancer research and treatment. It displays annoying biases and inconsistencies which should have been corrected by careful editing. This book would have been ideal for trainees in medical oncology, hematology, urology, or radiation therapy who wish to have an in-depth review of the literature in this important and rapidly changing field.

Nicholas J. Vogelzang, MD
Joint Section of Hematology/Oncology
University of Chicago Medical Center
Chicago, IL

December 10, 1986

ANNOUNCEMENTS

National Cancer Institute Semicentennial: NEW DATE

The NCI will observe its 50th anniversary in 1987. To celebrate the occasion, the Institute will hold an alumni reunion 4–7 PM on May 26, 1987, at the Bethesda National Naval Medical Center. All past employees, as well as present staff, will be welcome.

Alumni are invited to write for further details to: B. H. Morrison, III, MD, Bldg 31, Rm 10A52, National Cancer Institute, Bethesda, MD 20892.

Principles of Epidemiology

Chicago, Illinois, April 27–29, 1987, and Washington, DC, October 28–30, 1987

For further information, contact: The New England Epidemiology Institute, Department SC-4, PO Box 57, Chestnut Hill, MA 02167 (telephone: 617-734-9100).

Seventh Annual Epidemiology Summer Program

Tufts University, Medford, Massachusetts, July 26–August 14, 1987

For further information, contact: The New England Epidemiology Institute, Department SC-4, PO Box 57, Chestnut Hill, MA 02167 (telephone: 617-734-9100).

2nd International Symposium on Tissue Repair

Tarpon Springs, Florida, May 13–17, 1987

For further information, contact: Symposia Medicus, 2815 Mitchell Dr, Suite 108, Walnut Creek, CA 94598 (telephone: 415-935-7889).

First International Symposium on Organ Directed Toxicities of Anticancer Drugs

Burlington, Vermont, June 4–6, 1987

For further information, contact: Miles P. Hacker, PhD, Vermont Regional Cancer Center, 1 South Prospect St, Burlington, VT 05401 (telephone: 802-656-4414).

Third International Conference on Malignant Lymphoma: Current Status and Prospects

Lugano, Switzerland, June 10–13, 1987

For further information, contact: F. Cavalli, MD, Division of Oncology, Ospedale San Giovanni, 6500 Bellinzona, Switzerland (telephone: 091-23-62-10).

International Society of Paediatric Oncology—SIOP XIX Meeting

Jerusalem, Israel, June 14–19, 1987

For further information, contact: Prof. Rina Zaizov, Chairperson, PO Box 983, Jerusalem 91009, Israel.

8th International Symposium on Carotenoids

Boston, Massachusetts, July 27–31, 1987

For further information, contact: Dr. Richard F. Taylor, 8th International Symposium on Carotenoids, c/o Applied Biotechnology, Arthur D. Little, Inc, Acorn Park, Cambridge, MA 02140.

International Gynaecological Oncology Meeting

Charing Cross Hospital Medical School, London, England, September 2–3, 1987

For further information, contact: Dr. Newlands, Charing Cross Hospital, Fulham Palace Rd, London W6 8RF, England (telephone: 01-748-2040).

First International Paediatric Oncology Nursing Meeting

Jerusalem, Israel, September 13–18, 1987

For further information, contact: Prof. Rina Zaizov, Chairperson, PO Box 983, Jerusalem 91009, Israel.

Second International IST Symposium: Multimodality Treatment of Ovarian Carcinoma

Genoa, Italy, September 24–26, 1987

For further information, contact: P. F. Conte, MD, Scientific Secretariat, Istituto Nazionale per la Ricerca sul Cancro, V. le Benedetto XV-10, 16132 Genoa, Italy (telephone: 010-352828/352803).

2nd International Congress on Neo-Adjuvant Chemotherapy

***Palais des Congrès*, Paris, France, February 19–21, 1988**

For further information, contact: S. O. M. P. S., Hôpital de la Salpétrière, 47 Boulevard de l'Hôpital, 75651 Paris Cédex 13, France.

CORRECTION

In the article "High-Performance Liquid Chromatographic Assay for Taxol in Human Plasma and Urine and Pharmacokinetics in a Phase I Trial" by S. M. Longnecker, R. C. Donehower, A. E. Cates, T.-L. Chen, R. B. Brundrett, L. B. Grochow, D. S. Ettinger, and M. Colvin (Cancer Treat Rep 71:53–59, January 1987), the chromatographic separations depicted in figures 3 and 4 (page 56) are transposed. The tracing shown at the top of the column should appear with the legend for figure 4, and the tracing below should appear with the legend for figure 3.

Cancer Treatment Reports

COMMENTARY

229 Reappraisal of Some Dosage Adjustment Guidelines

ORIGINAL ARTICLES

235 Transnodal Cancer Chemotherapy for Metastatic Lymph Nodes in Rats

241 Synergy Between Low-Dose Chemotherapy and Immunotherapy in Mouse L1210 Leukemia

247 Potentiation of Cytotoxicity of Zinostatin Under Acidic Conditions In Vitro and In Vivo

253 Weekly 5-Fluorouracil Combined With PALA: Toxic and Therapeutic Effects in Colorectal Cancer

257 Local Neurotoxicity After Intra-arterial Cisplatin in Head and Neck Cancer

261 Thymidylate Synthase Gene Amplification in a Colon Tumor Resistant to Fluoropyrimidine Chemotherapy

267 Experimental Dacarbazine Antitumor Activity and Skin Toxicity in Relation to Light Exposure and Pharmacologic Antidotes

273 Phase I Study of Epirubicin Given on a Weekly Schedule

277 Second-Line Chemotherapy of Stage III–IV Ovarian Carcinoma: A Randomized Comparison of Melphalan to Melphalan and Hexamethylmelamine in Patients With Persistent Disease After Doxorubicin and Cisplatin

283 Chemoendocrine Therapy With Prolonged Estrogen Priming in Advanced Breast Cancer: Endocrine Pharmacokinetics and Toxicity

291 Small Cell Carcinoma of the Lung: Influence of Age on Treatment Outcome

297 Use of Nude Mouse Xenografts as Preclinical Drug Screens: In Vivo Activity of Established Chemotherapeutic Agents Against Melanoma and Ovarian Carcinoma Xenografts

305 Phase II Trial of Etoposide in APUD Tumors

BRIEF REPORTS

309 Continuous-Infusion Diaziquone in Acute Myeloid Leukemia: A Southeastern Cancer Study Group Trial

311 Phase II Study of Carboplatin in Previously Untreated Patients With Metastatic Small Cell Lung Carcinoma

313 Percutaneous Hepatic Arterial Infusion of Cisplatin for Metastatic Breast Cancer

317 Pharmacokinetic Comparison of Intranasal, Oral, and Intramuscular Metoclopramide in Healthy Volunteers

CLINICAL TRIALS SUMMARIES

321 Phase II Study of Etoposide in Previously Untreated Squamous Cell Carcinoma of the Head and Neck

323 Phase II Study of Aclarubicin in Non-Small Cell Lung Cancer

325 Phase II Trial of Esorubicin in Patients With Advanced Melanoma

327 Treatment of Recurrent or Metastatic Poorly Differentiated Adenocarcinoma of the Endometrium With Tamoxifen and Medroxyprogesterone Acetate

329 Phase II Trial of Cisplatin and Etoposide in Adenocarcinomas of the Upper Gastrointestinal Tract

331 Phase II Studies of PCNU and Bisantrene in Advanced Renal Cell Carcinoma

333 Phase II Clinical Trial of Tricyclic Nucleoside Phosphate for Advanced Colorectal Cancer

335 Phase II Study of Vinblastine and Bleomycin in Advanced Ovarian Cancer

NIH Publication No. 87-22

March 1987